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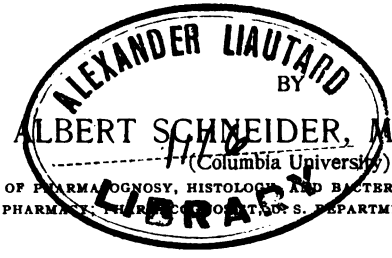
# PHARMACEUTICAL BACTERIOLOGY

SCHNEIDER

# PHARMACEUTICAL BACTERIOLOGY

WITH SPECIAL REFERENCE TO

DISINFECTION AND STERILIZATION




BY

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WITH 86 ILLUSTRATIONS

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# PREFACE.

The recent growth and development of the professional side of pharmacy has made new text-books necessary. The present volume is the product of such progress.

The illustrations have been selected with a view to a fuller explanation of the text. The descriptions of the illustrations have been made unusually complete. This is to make it possible for the student to ascertain the use of every article illustrated without the necessity of searching for additional information in the text itself. Some of the illustrations are from original drawings, others are from electros supplied by the Bausch & Lomb Optical Company and the Cutter Biological Laboratory of Berkeley, California. Still others are taken from recent works on bacteriology, notably Williams' "Manual of Bacteriology."

Attempts have been made to adhere strictly to the subject from the standpoint of the pharmacist, with only enough treatment of general bacteriology to make clear the collateral relationships, especially as it pertains to medical and commercial or industrial bacteriology.

While this volume is primarily intended for students in colleges of pharmacy, it is hoped it will also be found useful by practising pharmacists.

SAN FRANCISCO.

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# PHARMACEUTICAL BACTERIOLOGY.

## CHAPTER I.

### GENERAL INTRODUCTION.

In introducing the first of a new series of text-books, certain explanations are necessary or at least desirable, which, after the subject is well established, become superfluous. Comparatively speaking, the science of bacteriology is not new, but its introduction into pharmacy is of very recent date.

Medical bacteriology forms the very framework of medical practice. It has brought about our modern antiseptic surgery which has been the means of saving countless lives. It has led to the still more recent discoveries in serum therapy and the opsonic theory of disease.

About 1896 a few of the colleges of pharmacy in the United States gave optional courses of instruction in bacteriology. At the present time nearly all of the leading colleges of pharmacy give instruction in bacteriology and in many of these institutions the courses are compulsory, forming a part of the prescribed curriculum, represented by lectures and laboratory work. In some universities the students of pharmacy receive their bacteriological instruction in the department of medicine or perhaps dentistry. However, pharmaceutical bacteriology and medical bacteriology are quite distinct. Medical students study this subject from the standpoint of pathology and disease, matters which concern the pharmacist but little. Students of pharmacy do not have the time necessary to devote themselves extensively to special laboratory methods and technic, nor is it advisable that they should receive extensive laboratory instruction in pathology. Pharmaceutical bacteriology must be suitably adapted to the practice of pharmacy.

The pharmacist should have a fair knowledge of general bacteriology, in order that he may realize what important relationships bacteria bear to human activities in general, to medical practice more especially, and in order that he may comprehend quite fully the significance of these minute organisms in pharmaceutical practice. He should know what pharmaceutical preparations and what medicinal substances are likely to be attacked by bacteria, and what changes they are capable of producing in such substances. He

should have some knowledge of the effects that bacterially deteriorated substances may have when introduced into the human organism. He should be qualified to sterilize pharmaceuticals as is now required in the pharmacopoeias of several foreign countries as Austria, Italy, and Belgium. He should know something of the comparative value of the numerous disinfectants and antiseptics used and found upon the market and should know how to standardize these agents according to recent bacteriological methods. The pharmacist should know that bacteria, yeasts, and related organisms develop very promptly and profusely in all aromatic waters; in carelessly manipulated boiled and distilled water; in dilute solutions of all acids and alkalies; in dilute alcohol and alcoholic liquids; tinctures; infusions; extracts, solid and liquid; decoctions; in dilute salt solutions; in plant juices; mucilages; emulsions; elixirs; wines; in syrups of all kinds; in carelessly manipulated vegetable drugs, crude and powdered; in drugs from the animal kingdom, as ox-gall, lard, oils, fats, pepsin, etc. He should have a clear comprehension of antiseptics as germ destroyers, and should know how to prepare and use them. He should have a general knowledge of alimentary and systemic phagocytosis; of leucocytosis in inflammatory processes, in pus formation, necrosis, etc. He should comprehend immunity, natural and acquired; he should know about opsonins and the opsonic index. He should have a general knowledge of bacterial enzymes; of toxins, ptomaines, leucomaines; of antitoxins; of bacterial vaccines. He should have a special knowledge of the source, manufacture, and use of antitoxins and toxins, modified toxins, vaccine virus, and related products used in medical practice. He should have a general knowledge of the causation of the more common bacterial and protozoic diseases. He should have special instruction in the disinfection of public and private dwellings, and should be able to coöperate with the physician in stamping out threatened epidemics and in carrying out public prophylactic and hygienic measures. To attain these ends a knowledge of bacteriology, specialized to suit the needs of the pharmacist, is absolutely essential.

It is not the so-called practical side of bacteriology, represented by dollars and cents, which should interest the pharmacist in this science, but rather the broader view of his profession which will enable him to perform his duties more intelligently and more efficiently. The man whose actions are altogether prompted and directed by the dollar sign has no place in pharmacy or in medicine. He should turn to some non-professional enterprise.

As yet there are no text-books or other works devoted especially to pharmaceutical bacteriology. Text-books on bacteriology for use in universities, medical colleges, and technical schools are not suitable for use in colleges of pharmacy. Some of these books are excellent collateral reading for pharmacists, but most of them are of such a highly specialized nature that they

would no doubt do more harm than good should the average pharmacist attempt to use them as a practical guide in the performance of his duties. Bacteriology must not be made discouragingly difficult to the pharmacist, in order that the best results may be attained.

Wherever possible the college instruction in pharmaceutical bacteriology should be supplemented by visits to biological laboratories for the manufacture of sera and bacterial vaccines, to board of health laboratories, quarantine stations, garbage reduction works, etc. Students should also be assigned special reading. Journals and special treatises on bacteriology and on public sanitation should be consulted. The reports on bacteriological and related subjects issued from time to time by the United States Public Health and Marine Hospital Service are of special interest.

The following references are given for the benefit of those students who may desire further information regarding the earlier conceptions of pharmaceutical bacteriology. It will be found that the opinions advanced by the authors cited differ considerably.

1. Bacteriology for Pharmacists. *Pharm. Journ. Trans.*, 23 (III), 565, 865; 24 (III), 101, 1893.

Largely a description of the apparatus employed in bacteriological work, giving special attention to the value and use of the compound microscope in such work.

2. H. P. Campbell. Bacteria Dangerous to Medicines. *Am. Journ. Pharm.*, 72, 113–118, 1890.

3. R. G. Eccles. Pharmaceutical Bacteriology. *Proc. A. Ph. A.*, 42, 225–230, 1894.

A very interesting paper on the theoretical possibilities of pharmaceutical bacteriology.

4. J. L. Hatch. Bacteriology. *Pharm. Journ. Trans.*, 22 (III), 271, 289, 330, 1891.

A series of lectures delivered before the alumni association of the Philadelphia College of Pharmacy, devoting the major attention to the morphology, physiology, and classification of bacteria.

5. R. T. Hewlett. Bacteriology in its Practical Aspects. *Pharm. Journ. Trans.*, 25 (III), 819–820, 893–894, 1895.

A general retrospect of bacteriology as a possible source of financial gain to the pharmacist.

6. Smith Ely Jeliffe. Moulds and Bacteria. *Druggists Circular*, 94–95, 1897.

A description of some of the more common moulds and bacteria found in medicinal solutions. Good illustrations.

7. E. Klein. Bacteria, Their Nature and Function. *Pharm. Journ. Trans.*, 23 (III), 15, 35. 1893.

8. W. H. Lymans. Bacteriological Culture Apparatus. *Pharm. Journ. Trans.*, 1893. (*National Druggist*, 173, 1893.)

9. Albert Schneider. Pharmaceutical Bacteriology. *Proc. A. Ph. A.*, 48, 186–189, 1894.

10. Specialism in Pharmacy, begotten by Progress in Bacteriology. *Pharm. Journ. Trans.*, 25 (III), 625, 1895.

Points out the necessity of a suitable preparatory training; the importance of a knowledge of the use of antiseptics.

11. R. Warrington. The Chemistry of Bacteria. *Pharm. Journ. Trans.*, 23 (III), 402, 1893. (*Pharm. Era.*, 104, 1894.)

## CHAPTER II.

## HISTORICAL.

It must be evident that the science of bacteriology had its inception with the discovery of the compound microscope. For some time the progress in bacteriological investigation continued parallel with the progress in the mechanical perfection of the microscope and with the advance in microscopical technic. Gradually, however, the chemical and physiological investigations pertaining to bacteria gained in importance and significance. Our knowledge of the morphology of bacteria as revealed through the compound microscope has been practically stationary for two decades, but not so our knowledge of bacterial products and bacterial action. The methods of bacteriological technology have been gradually perfected, and the progress along this line has kept pace with the chemical and physiological investigations.

Although, as indicated, the science of bacteriology is of comparatively recent origin, yet we must not lose sight of the fact that many of the ideas underlying this science, as now comprehended, were advanced in remote antiquity. For this reason it is desirable to set forth these earlier concepts in a historical review. Most of the writers on general bacteriology, who make reference to the history of the subject, almost invariably mention the older ideas regarding spontaneous generation as being the forerunners of the modern ideas of bacteriology. It is, however, the ancient theories and beliefs pertaining to the cause of decay, disease, and epidemics which are even more directly associated with the first more important discoveries pertaining to modern bacteriological pathology.

For the purposes of simplification, condensation, and greater clearness the historical review is divided into periods or epochs. It is not possible, in the following brief outline, to cite all investigations of importance. Only a few of the epoch-making specialists are mentioned.

### Period I.

**From Hippocrates (300 B. C.) to Leeuwenhoek (1656). (The earliest ideas regarding epidemics and spontaneous generation.)**

From the earliest times the more scholarly writers mentioned certain noxious gaseous, and odoriferous substances or effluvias as being the cause of epidemics. These effluvias were supposed to emanate from the soil, from

the air, from water, stagnant pools, marshes, from decaying and putrescent substances, from crowded habitations, army camps, etc. The common people throughout the world and throughout all ages have held the belief that pestilence and disease was the manifestation of divine or supernatural influence, the judgment of an angry deity, a punishment inflicted on mankind for their sins and iniquities, beliefs which are occasionally asserted even at the present time. Changes of season, climatic conditions, and the influence of heavenly bodies were also considered as causative of diseases of an epidemic nature.

Animals, such as rats, mice, and insects, have long been recognized as possible carriers of disease. An English investigator has recently discovered some very excellent sanitary rules in the Vedas of the Hindus. The following is a translation from Book VI, verse 50, of the Atharva-Veda.

"Destroy the rat, the mole, the boring beetle; cut off their heads, O asvins.

"Bind fast their mouths; let them not eat our barley; so guard ye twain our growing corn from danger.

"Hearken to me, lord of the female borer, lord of the female grub! Ye rough-toothed vermin.

"Whate'er ye be, dwelling in woods, and piercing, we crush and mangle all those piercing insects."

By "piercing insects" no doubt mosquitos are meant. If the injunctions were literally obeyed, plague, malaria, and certain protozoic diseases would be abolished from India.

Hippocrates (460–377 B. C.), the father of medicine, considered seasons and winds as the cause of pestilence, particularly the long continued southerly winds (for Greece), and a warm, humid, clouded atmosphere. Galen (130–220 A. D.) held similar beliefs. He declared that diseases arose from a putridity of the air or from atmospheric and weather conditions. Marcellinus (359 A. D.), a warrior as well as philosopher and historian, declared that the decomposing bodies left on the battlefield were the cause of "pestilential distempers," also caused by extremes in weather, by marsh effluvias, violent heat, and a vitiated atmosphere. Aetius (fifth century), an eminent physician, declared that epidemics or common diseases were caused by bad food, bad water, immoderate grief, hunger, excesses, particularly abundance following extreme want, lack of exercise, excessive humidity, and putrid substances. Alpinus, a Venetian physician of the sixteenth century, explained how the cause of plagues and epidemics may be carried by persons or in cargoes. He pointed out that a given disease from one country is more malignant than the same disease from another country. During the dark and middle ages various ecclesiastical and lay writers ascribed epidemics and pestilence to a variety of causes—the wrath of God, to demons or evil spirits, comets, meteors, earthquakes, volcanic eruptions, cyclones, eclipses of the sun, terrific storms, wars, famines, great fires, etc. Even as late as 1799

no less an authority than Noah Webster makes the following declaration: "All the great plagues which have afflicted mankind have been accompanied with violent agitations of the elements. The phenomenon most generally and closely connected with pestilence is an earthquake. From all the facts which I can find in history, I question whether an instance of any considerable plague, in any country, can be mentioned which has not been immediately preceded by, or accompanied with, convulsions of the earth. If any exceptions have occurred, they have escaped my researches. It does not happen that *every place* where pestilence prevails is shaken; but during the progress of the disease which I denominate *pestilence*, and which runs, in certain periods, over large portions of the globe, some parts of the earth, and especially those which abound most with subterranean fire, are violently agitated." Were Noah Webster alive, he would certainly cite the recent plague on the Pacific Coast as bearing out his assertions. On April 18, 1906, the coast region about San Francisco was certainly "violently agitated," and this phenomenon was followed by the plague (black pest, bubonic plague). But what were the actual facts? The plague had, in all probability, existed in a sporadic form in "Chinatown," in San Francisco, and in other places on the Pacific coast for many years. In 1903 several authentic cases came to notice and were reported. The reasons why the disease had not previously gained a stronger foothold in San Francisco are several. Chinatown is more or less isolated (socially, at least) from the rest of the city, and the poorer, more filthy class of the Chinese do not as a rule mingle with the white population. The disease is an Oriental filth disease. After the earthquake and fire of April 18–22, 1906, the Chinese of all classes, the plague-infected rats and fleas of the Chinese quarters, became thoroughly intermingled with the rest of the stricken population, and as a result there were established several new foci of plague infection, which accounted for the increase in plague cases in 1907, a condition which was soon under control, thanks to the strenuous efforts of the federal government, the board of health, and various citizens' organizations.

Several writers of remote times, as well as occasional writers of the dark and middle ages, held the opinion that the cause of disease, the disease-producing effluvias, might be carried long distances by air currents, in ships, or by caravans, and that the poison may enter the system *via* the air passages, through the skin, or through the digestive tract. Hodges, an Englishman, who wrote a treatise on the London plague of 1665, declared that some essential alteration in the air is necessary to the propagation of this disease. That is, the "nitro-aerial" principle, which causes or invigorates plant and animal life, is supposed to become vitiated.

The corrupting principle is a "subtle aura or vapor" which is "extricated from the bowels of the earth." This plague-causing poison was said to

affect trees and other plants, fishes and other animals, as well as man. Dr. Mead declared that epidemics were caused by (1) diseased persons, (2) goods imported from infected places, and (3) a vitiated or poisoned state of the air, notions which may be considered as the direct forerunners of the germ theory of disease.

Let us now go back and consider the ancient ideas regarding spontaneous generation. Anaximander, of Miletus, who lived during the forty-third Olympiad (610 B. C.), believed that many animals developed *de novo*, from moisture and water acted upon by sun and warmth. The extremist, Empedocles of Agrigentum (450 B. C.), declared that all living things upon the earth were capable of originating spontaneously. Aristotle (384 B. C.) taught that *some* plants and animals originated spontaneously. Ovid, some

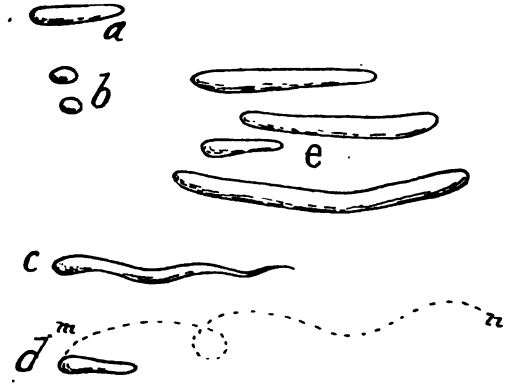


FIG. 1.—From the *Arcana Naturæ* of A. van Leeuwenhoek. The first published illustration of bacteria. These bacilli of the mouth cavity were seen with the aid of simple lenses only. *a*, *b*, bacilli; *c*, a spirillum; *e*, perhaps chain forms of bacilli; *d*, illustrating the characteristic motion of certain bacilli (*n* to *m*).

three centuries later, gives instructions how to create bees spontaneously in the carcasses of horses. To within recent times the belief that certain animals could originate spontaneously, that is, without a pre-existing parent, was quite general, and differed only in grotesqueness. Cardan as late as 1542 declared that water created fishes, and that many fermentative processes created animals. Van Helmont gives instructions how to produce mice artificially. Kircher boldly declared that he had seen certain animals develop spontaneously before his eyes. Paracelsus gives instructions how to make homunculi. The instructions are quite simple. Certain substances are placed in a bottle, the bottle is well stoppered and buried in a manure heap. Every day certain incantations must be pronounced over the bottle in the manure heap. In time, Paracelsus declared, a small living human being (homunculus) will appear in the bottle. Paracelsus, however, naïvely

admits that he has never succeeded in inducing the homunculus to continue alive after being taken from the bottle. Gradually these grotesque and extreme opinions regarding spontaneous generation were abandoned, and it was declared that only the lower plants and animals, such as seaweeds, algæ, lichens, lice, mites, maggots, etc., could develop spontaneously. In fact, we can find fairly intelligent individuals to-day who firmly believe that certain animals, as lice, mites, etc., can originate without a parent, and that the hair from the tail or mane of a horse will change into a worm or snake if placed in a bottle of water and exposed to light and warmth.

From the earliest records we learn that the value of disinfectants in preventing the spread of infectious diseases (epidemics and plagues) was known. Ovid states that the shepherds of his time used burning sulphur for bleaching wool and to free it from infectious diseases. In times of plagues, big fires were made to stay the ravages of pestilential diseases. The Mosaic law is replete with instructions regarding cleanliness as a means of preventing disease. Wine was highly valued as a dressing for wounds, having the effect of preventing or checking pus formation.

## Period II.

**From Leeuwenhoek (1656) to Schwann (1837). (Discovery of micro-organisms and the early investigations regarding their activities.)**

As early as 1646 Kircher suggested that certain diseases might be due to very minute organisms which were supposed to originate spontaneously

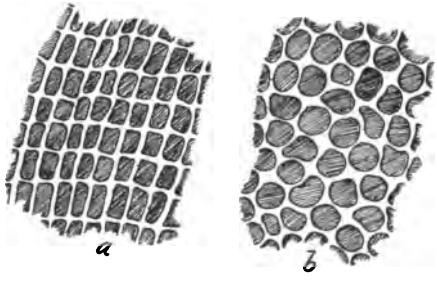


FIG. 2.—From *Arcana Naturæ*. Cell structure of cork. Cell-lumen is shaded and cell-walls are shown light.

under certain conditions. Anton van Leeuwenhoek is very justly called the father of microscopy, and to him must undeniably be given the credit of first having discovered and actually figured microbes and other micro-organisms. His *Arcana Naturæ* was published in 1656 in four volumes. It is a most interesting work, and contains many good illustrations showing

microbes of the mouth cavity, infusoria of stagnant water and cellular structure of vegetable tissues. He observed the motion of bacteria and infusoria, made measurements, illustrated capillary circulation in the web of the frog's foot, etc. He was closely followed by Robert Hooke, who published his *Micrographia* in 1658. The discoveries of Leeuwenhoek and Hooke were certainly epoch-making. A new world of minute organisms was made known, the question of spontaneous generation received a new turn, and the way to the discovery of the causes of disease and fermentation was paved. In 1660 Leeuwenhoek discovered yeast cells. From 1660 to 1760 the microscope was actively employed by a few investigators, and additions were slowly made to the list of micro-organisms. Audry (1701) designated microbes worms. Müller, of Copenhagen (1786), grouped them under two divisions, *monas* and *vibrio*. In 1743 Henry Baker, of England, published his work, "The Microscope Made Easy," from which it would appear that very little progress had been made since the time of Leeuwenhoek (1656).

As early as 1686 Franceso Redi doubted that maggots were generated *de novo* in putrid meats. He noticed that the presence of the maggots was preceded by swarms of flies which, he concluded, had something to do with the development of the maggots. He found that meat from which the flies were excluded by means of paper or a very fine mesh wire screen, simply decayed without any development of maggots. The paper cover and the fine screen kept the eggs of the flies from being deposited on the meat, and the meat was not infested by maggots, which, as Redi rightly conjectured, developed from the eggs of the fly-like imago. This very simple but reliable experiment did much to create doubt as regards the correctness of the theory of spontaneous generation and other related beliefs.

Spallanzani (1777) was among the first to demonstrate experimentally that boiling and hermetically enclosing fermentable liquids prevented fermentation. Ehrenberg (1828) discovered microscopic organisms in dust and in water, and in 1833 he classified all known bacteria under four orders, bacterium, vibrio, spirillum, and spirocheta. Cagniard-Latour and von Schwann (1836) discovered the vegetable nature of yeast, and in 1837 Schwann decleared that yeast was the direct cause of fermentative changes resulting in the liberation of alcohol and $CO_2$, and that the causes of decay were to be found in the atmosphere. Berzelius (1827) declared that the yeast cells were the direct cause of fermentation. F. Schulze (1836) prevented decay in liquids containing certain organic substances by first heating or boiling them and excluding the air by means of a layer of oil or by closing the container with cotton and supplying it with air which had been sterilized by passing through sulphuric acid. Braconnot (1831) advanced the theory that yeast cells had the power of holding, and condensing within the

cell-substance, the oxygen of the air and conducting it to the substances undergoing fermentation, resulting in the splitting up of sugar into alcohol and carbonic acid gas.

The question of spontaneous generation was again discussed with renewed energy. The belief that larger animals could originate *de novo* was quite generally abandoned, but it was very persistently argued that micro-organisms, maggots and a few other very small animals could thus develop. Bastian was perhaps the leader in the arguments in favor of spontaneous generation, opposed by Schwann, Pasteur, and others. Schroeder and von Dusch demonstrated that decay could be prevented by boiling and supplying air that had been filtered through cotton. Pasteur (1862) used bent tubes to supply air to the previously sterilized (by heating) substance, as shown in Fig. 3.

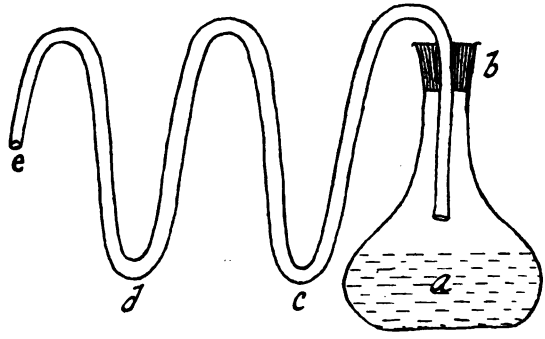


FIG. 3.—Flask, containing an organic substance, *a*, hermetically closed by means of a stopper, *b*. The bent tube is open at *e*, admitting air. Dust and microbes lodge at the bends *d* and *c*.

The microbes in the air passing through the tube are deposited (by gravity) in the lower bends of the tube. Those favoring the theory of spontaneous generation nevertheless continued their arguments. It was pointed out that changes of decay took place in eggs, in internal tissues and organs of the dead as well as in the living, etc., where, it was supposed, microbes could not possibly have access. However, further convincing experiments gradually silenced all opposition. Bastian and a few followers took practically their last stand in 1875, and since that time no scientist of repute has ever argued in favor of spontaneous generation, though the question of the primal origin of living things remains unanswered.

Vaccination as a protection against virulent small-pox was practised early in the eighteenth century in Turkey and other Oriental countries, and was introduced into Europe *via* England through the influence of Lady Mary Wortley Montagu. A. von Humboldt states that the Mexicans practised vaccination at a very early period. This early vaccination mate-

rial was obtained from a pustule of a small-pox patient, and not from the cow, as at present. The immunity against subsequent attacks was established, but the disease transmitted through this older method of vaccination was severe and often fatal; besides, the general vaccination was a source of spreading the disease. In 1840 this form of vaccination was prohibited in England by act of Parliament.

In 1768 Jenner's attention was attracted to the value of vaccination, and after a series of patient researches he perfected the method of vaccination by means of the virus obtained from a cow which had been inoculated with small-pox (vaccinia). Jenner established the first public institution for vaccination in 1799, and in the following year the practice was introduced into France, Germany, and the United States. Vaccination with vaccinia material is now universal in all civilized countries and in countries under civilized control, and as a result small-pox in an epidemic form does not occur in these countries, and the disease has become less and less virulent, so that it is no longer the dreaded scourge that it was two centuries ago. In spite of the beneficent influence of vaccination, there are individuals who oppose this simple, harmless operation with all the energy that ignorance is capable of. Civilized countries are beginning to raise the long-enforced small-pox quarantine as a wholly unnecessary infliction, because vaccination makes the spreading of small-pox impossible. France has raised the quarantine, and so have several other countries, examples which will no doubt soon be followed generally. In conclusion, it is of interest to note that the primary cause of small-pox is unknown even to this day. No organism has thus far been isolated from diseased tissues to which small-pox manifestations could be ascribed.

## Period III.

**From Schwann (1837) to Pasteur (1862). (Investigations pertaining to the relationship of micro-organisms to fermentation and disease.)**

The discoveries of the cause of fermentation, of decay, and of wound infection are closely associated. Many centuries ago Varro expressed it as his opinion that certain minute animals, breeding in marshy places, got into the system through mouth and nostrils and caused the disease and decay of tissues. Theodoric (1260) taught that wound infection came from the air. To prevent such infection he applied wine, which is known to be somewhat antiseptic. John Colbach (1704) described a "new and secret method of treating wounds by which healing took place without inflammation or suppuration."

From earliest time up to as late as 1860, it was quite generally taught

that all normal healing of wounds and cuts must be preceded by pus-formation. A "laudable pus" was recognized, the presence of which was looked upon as a hopeful sign and indicated that repair was proceeding favorably. If the laudable pus which was of a whitish creamy consistency changed to a watery consistency, it was considered an unfavorable sign.

After Schwann and others had demonstrated that fermentation was due to the presence of yeast cells, and it was proven conclusively that decay was caused by bacteria, the relationship of bacteria to disease began to receive consideration. Rayner and Devaine (1850) found bacterial rods in animals suffering from splenic fever. As early as 1840 Henle, who is by some considered the father of modern bacteriology, made some very valuable deductions regarding the relationship of micro-organisms to disease. He recognized a "contagium" (the active cause of the disease associated with micro-organisms), which was supposed to be air-like and yet at the same time fixed. It was supposed to retain its activity for years in the dry state. An unweighable and unmeasurable quantity of this substance may cause an extensive epidemic. Air currents can carry the contagium great distances and cause epidemics in widely separated areas. Bassi (1835) declared that a fungus was the cause of the muscardine disease of silkworms. Pollender (1855) reported that bacteria caused anthrax, verified by Devaine in 1863. Hallier, an enthusiast but not reliable as an investigator, declared that scarlet fever, measles, typhus, and cholera were caused by bacteria. His deductions were, however, not based upon scientific research and proof. Rindfleisch (1866) and Waldeyer (1868) gave considerable attention to wound infection, which, they declared, was due to microbic invasion. In 1869 Pasteur demonstrated the microbic cause of the silkworm disease which interfered very seriously with the silk industry in France. Pasteur and Klebs demonstrated experimentally that bacteria could be grown in artificial culture media, and Robert Koch proved that the pathogenic microbes actually secreted the disease-causing substance. This was demonstrated by transferring an infinitely small quantity of the germ material from a diseased organ to a suitable culture medium and making sub-cultures, until the last culture must contain less than the trillionth part of the original substance. Nevertheless, inoculations from the last culture developed the disease with full energy. This experiment was made to meet the assertions that the cause of the disease did not reside in the bacterium, and that the bacterium, if present in the disease, was merely incidental to and not causative of the disorder.

A heated controversy continued for some time. Such authorities as Liebig, Nägeli, Bastian, Cohn, Billroth, Hiller, Schroeder, Hoppe-Seyler, Kühne, Tiegel, Sanderson, Nencki, Serval, and Paschutin declared that micro-organisms were not the cause of decay, fermentation, and disease;

that these changes were due to chemical substances. However, such men as Pasteur, Koch, Panum, Klebs, and others forged link after link in the chain of evidence connecting the causative relationship of bacteria to disease.

## Period IV.

**From Pasteur (1862) to Behring (1890). (Period of remarkable activity in pathological bacteriology.)**

It would be impossible in a brief review to cite all of the important investigations of this period. Pasteur, Koch, and others had already given the subject of bacteriological technic considerable attention. The most suitable culture media, laboratory apparatus, stains, etc., were determined. The compound microscope had now reached a high degree of perfection, and the oil-immersion lenses made the closer study of the morphology of bacteria possible.

As might be expected, the importance of germicides in surgery received first attention. The "laudable pus-" formation ideas were abandoned. It became the surgeon's duty to induce "primary union" or healing by "first intention," that is, healing without any pus formation whatever. This demanded that the surfaces of the incision be brought in close contact, and that all bacterial infection be prevented by the use of antiseptic dressings, antiseptic solutions in the form of irrigations and sprayings, etc. Sir Joseph Lister, of Scotland (1875), brought the use of disinfectants in surgery to a high degree of perfection, and modern antiseptic surgery is often designated "Listerism." The modern proprietary antiseptic "listerine" is named after this eminent surgeon. The chief antiseptic of Lister and his followers was carbolic acid, which was used for free wound irrigation and general disinfection. He operated in a spray of carbolic acid solution. As late as 1890 there was to be found an occasional lecturer in a college of medicine who held out against the germ theory, and not a small number of the eminent opponents mentioned in the previous period carried their mistaken notions with them to the grave.

The name of Robert Koch will stand throughout the ages as the leader in modern bacteriological science. Early in life he was convinced of the correctness of the germ theory of disease, but his first contributions to bacteriological science awakened a storm of opposition. Billroth, of Vienna, and others persisted in declaring that microbes were not causative of pus-formation or of the development of disease; but that microbes might be accidentally present, due to the action of a "phlogistic zymoid" which developed in the animal organism.

In 1882 the French government sent a medical commission to India to determine if possible the cause of Asiatic cholera, but the commission re-

turned with a negative report as far as a bacterial cause of the disease was concerned. In 1883 the German government sent a similar commission, headed by Robert Koch, and the report of this commission was that Asiatic cholera was caused by a bacillus, the famous comma bacillus of Koch. The work of Koch in connection with the study of cholera seemed to act as a wonderful stimulus, and other eminent investigators made important discoveries within the year or two following. Klebs and Löffler discovered the diphtheria bacillus in 1884. Fraenkel, Weichselbaum, and Friedländer discovered the pneumococcus in 1884. Nicolaier and Kitasato discovered the tetanus bacillus in 1884. Löffler and Schütz discovered the glanders bacillus in 1882, and the bacillus of hog erysipelas (Rothlauf) in 1885.

Pasteur in 1881 made his first experiments in reproducing rabies in susceptible animals by inoculation with material obtained from the spinal cord, medulla oblongata, and lobes of the brain of animals dead from rabies. In 1884 he reported his experiments pertaining to the modification of the virulence of rabies by successive inoculations into susceptible animals. His use of this modified rabies virus as a means of preventing a severe and fatal course of the disease in those bitten by animals suffering from hydrophobia, is familiar to all. Thousands of cases have been treated successfully at Pasteur institutes established throughout the larger cities of the civilized world.

The above are only a few of the important investigations of this period. The causative relationship of microbes to certain diseases was undeniably established. The voices of opposition were silenced.

This period is especially notable for the development of antiseptic surgery. As a result, operations were no longer dreaded as in former times. Fatal infections following operations now became rare. Thousands of lives are saved. To remove or destroy the pus germs in open wounds or to prevent the access of germs to wounds, cuts, and abrasions, has become a simple matter, a simple mechanical application of suitable antiseptics.

The progress of purely medical bacteriology was not so marked. Although it was proven that certain diseases were due to bacteria, there were no satisfactory means of destroying them in the system. Internal antiseptics were tried, but without satisfactory results, as a rule. However, preventive medicine based on a bacteriological knowledge gave good results.

## Period V.

**From Behring (1890) to Wright (1907). (Discovery of serum therapy, bacterial vaccines, and development of utilitarian bacteriology.)**

The subject of immunity from disease received early attention. Age

immunity, race immunity, animal immunity, individual immunity, artificial immunity, natural immunity, acquired immunity, etc., attracted attention and received careful consideration. Metchnikoff (1884) explained immunity on the supposition that certain white corpuscles (leucocytes, phagocytes) of the blood devoured the microbes which entered the system. These white blood corpuscles are the guardians of health. They attack and feed upon any disease germs which may enter the body, either *via* the digestive tract, the respiratory tract, or *via* the circulatory system. If the leucocytes are deficient in number, or if the microbes are excessive in number, disease will develop. This theory had numerous followers, as well as opponents. It is now generally accepted as correct, borne out by observation and by experimental evidence.

The next important discovery was that blood serum had bactericidal properties in a varying degree, and that in addition to this there was something in the blood which had a tendency to neutralize or destroy the action of the poisons or toxins formed by pathogenic microbes. No one particular bacteriologist can be said to have made these discoveries. We can only name a few of the leading investigators who worked along these lines, leading to the discovery of the relationship of immunity and antitoxins—Behring, Brieger, Buchner, Calmette, Chamberland, Ehrlich, Emmerich, Flügge, Fränkel, Hüeppe, Jetter, Kitasato, Klemperer, Löffler, Rankin, Roux, Wassermann, and others. These eminent authorities have demonstrated the possibility of developing or aiding the antitoxic or immunizing power of the blood or of the body cells by introducing sera obtained from the blood of animals in which the antitoxic power is naturally high or is made so as the result of special treatment. Numerous sera (containing antitoxins and toxins) were tried; the one which first proved entirely satisfactory was the diphtheria antitoxin of Behring, which is now in universal use. Others are used more or less successfully, and some are still in the experimental stage.

In 1890 Koch reported on a "lymph" to be used in the treatment of tuberculosis. This lymph was a glycerin extract of the toxin of the bacillus of tuberculosis, and was to be used in the treatment of this dread disease, but the hopes of Koch were not realized, as the remedy proved a failure, and it soon fell into disuse, to be again taken up very recently. In 1907 Wright made known his discovery of opsonins. According to this authority, there exist in the blood certain substances which have the power of acting on the invading bacteria in such a manner as to render them more liable to be attacked and assimilated by the white blood-corpuscles or leucocytes. There are possibly as many opsonins as there are microbes capable of being digested by the leucocytes. The microbe-devouring power of the leucocytes can be increased by the use of bacterial vaccines, which consist of suspensions

of microbes. Very minute quantities are injected into the system, and the resulting reaction increases the power referred to.

Toxins of bacterial origin received the attention of investigators, and antibodies (antitoxins) were extensively discussed as to their possible relationship to health and disease. Enzymes, in their relationship to life processes in plants and in animals, were investigated. It is now supposed that soil toxins of plant origin, as well as those of bacterial origin, influence plant growth. Glandular preparations (ductless glands) have been carefully tested, and several of these are in use.

As the result of Wright's discovery of the use of bacterial vaccines in increasing the opsonic index, the tuberculin (lymph) of Koch was again tried in the treatment of tuberculosis, apparently with some success.

It was found that there were many bacteria other than those which caused disease in animals and plants. Some were found to be decidedly beneficial. Bacterial cultures were employed in butter-making (ripening of cream), in cheese-making, in tanning, in paper-making, siloing, etc. Some bacteria are employed to exterminate certain pest animals. A microbic chintz bug exterminator was tried in 1895–97, but it proved a failure. Microbic rat and mice exterminators (azoa, ratite, mouratus, etc.) are now being tested, and they appear to be quite successful, at least in certain localities and under certain conditions. A microbic rabbit exterminator has been tried in Australia.

In 1879 Dr. Frank, of Berlin, began his investigations of the leguminous root nodule microbes. In 1893 the writer attempted to utilize these microbes in increasing the yield of certain gramineous crops. In 1896 Nobbe and Hiltner, of Germany, introduced a patented microbic fertilizer for leguminous plants. In 1907 a California soil microbe was isolated which appears to be especially active in promoting the growth of sugar beets. This experiment led to the supposition that perhaps every species of plant has its peculiar bacterial flora, symbiotically (mutually beneficent) associated with the root system, mutually essential to active development. The importance of soil bacteria in setting free plant foods has been demonstrated by numerous investigators of Europe and of the United States. Yeast and mould organisms are practically utilized in the manufacture of beer, saké, and other food and drink products.

The above condensed outline of the history of bacteriology may be summed up as follows:

1. Ancient conceptions of disease and of spontaneous generation, dating back to 500 years B. C.

2. Discovery of micro-organisms about 1660 by Leeuwenhoek, followed by the work of Robert Hooke and a few others.

3. Discovery of bacteria in air, dust, and decaying substances, and the

causal relationship of microbes to decay, and of the yeast organisms to fermentation.

4. Disproving the theory of spontaneous generation, by Schwann and others, about 1840.

5. Discovery of the bacterial origin of certain diseases—1862 to 1880.

6. Introduction of small-pox vaccination into England by Jenner in 1796.

7. Development of antiseptic surgery or Listerism—1875.

8. Period of great activity in pathological bacteriology—1880 to 1890.

9. Discovery of the causes of immunity to disease, antitoxin of diphtheria and other antitoxins, serum therapy, etc.—1886 to 1894.

10. Introduction of the use of certain bacteria in commerce and agriculture.

11. Discovery of opsonins and the use of bacterial vaccines. Reintroduction of Koch's lymph in the treatment of tuberculosis.

## Useful Works of Reference to Bacteriology and Related Topics.

The following references are selected for collateral reading. A few of these works are rare, and can be found only in some of the leading libraries. A reading of these and other related works will serve as a supplement to this text-book. It is not intended to imply that all of the works cited should be procured. Others besides those mentioned may be consulted as opportunity presents itself. Some of them can be obtained from public libraries; others may be ordered through the local book dealer, and a few may be borrowed from professional friends.

HENRY BAKER. The Microscope Made Easy. London. 1743.

Like the work of R. Hoke, this is of great historical interest, and is quite rare. Much of it is a copy of the work of Leeuwenhoek.

B. M. BOLTON (H. U. Williams). A Manual of Bacteriology. P. Blakiston's Son & Co., Philadelphia. 1910.

A most excellent work for medical students, also of value to students of pharmacy.

H. W. CONN. Agricultural Bacteriology.

This is a most excellent little work treating of bacteria in water, in the soil, in farm products, in the dairying industry, and in plants and domestic animals. It is well written in a simple, clear style.

H. W. CONN. Bacteria, Yeasts and Moulds in the Home. Ginn & Co. 1903.

This is of special value to the pharmacist, as the organisms described may also be found in pharmaceutical preparations.

H. W. CONN. The story of Germ Life. D. Appleton & Co., New York. 1905.

Very useful and interesting general reading on bacteriology.

S. M. COPEMAN. Vaccination, Its Natural History and Pathology. London. 1899.

Of historical interest, besides explaining the subject very fully.

E. M. CROOKSHANK. Text-book of Bacteriology. Philadelphia. 1897.

This is much used as a college text-book on bacteriological technic. Not especially adapted for general reading. Would serve as a laboratory guide.

CHAS. S. DOLLEY. The Technology of Bacteria Investigation. S. E. Casino & Co., Boston. 1885.

Good reference work on bacteriological technic. Somewhat out of date.

PAUL EHRLICH (Chas. Bolduin). Collected Studies on Immunity. John Wiley and Sons, New York. 1906.

An extensive discussion of the theories pertaining to the action of toxins and antitoxins. Ehrlich's side-chain theory is quite fully treated. The subject is too technical for the average reader, and is of great value only to the specialist in this branch of bacteriology.

DAVID ELLIS. Outlines of Bacteriology. London, New York and Calcutta. 1909.

An excellent English work on general bacteriology especially valuable from the technical and agricultural standpoints.

J. W. EYRE. The Elements of Bacteriological Technic. W. B. Saunders & Co., Philadelphia. 1902.

An excellent laboratory guide for the use of medical, dental, and technical students, and which will serve many purposes of the student of pharmacy.

DANIAL DE FOE. History of the Plague in London. London. 1857.

Of historical interest. Well written.

W. D. FROST. A Laboratory Guide in Elementary Bacteriology. Macmillan Company, New York. 1903.

An excellent laboratory guide. It contains no general information regarding bacteria, and can be used profitably only under the guidance of a laboratory instructor.

W. H. HARROCKS. An Introduction to the Bacteriological Examination of Water. J. A. Churchill, London. 1901.

Of value to anyone interested in the bacterial contamination of water supplies; also useful for general reading.

ROBERT HOOKE. Micrographia. London. 1665.

A very rare and very interesting work treating of the earliest discoveries through the use of the microscope. Some of the illustrations are excellent. Of great historical value and interest. Can be found only in a few of the larger university and public libraries. In English.

L. O. HOWARD. Mosquitoes: How They Live and How They Carry Disease. McClure, Phillips & Co., New York. 1901.

Contains valuable information regarding these pests and how they carry diseases. Of special value in yellow fever and malarial districts.

E. O. JORDAN. A Text-book of General Bacteriology. W. B. Saunders & Co., Philadelphia. 1908.

For medical students. Contains much information of interest to the pharmacist.

F. LAFAR (Salter). Technical Mycology. London. 1903.

Rather technical for general reading. Treats of fermentation and fermentation products, use of yeast organisms and bacteria in the industries, etc. Especially valuable to those interested in beer-making, etc., the dairying industry, etc.

MILLARD LANGFELD. Infectious and Parasitic Diseases, Including Their Cause and Manner of Transmission. P. Blakiston's Son & Co., Philadelphia. 1907.

Contains much valuable information on preventive medicine, sources of infection, disinfectants and disinfection, animal parasites, etc. Excellent collateral reading for the pharmacist.

ANTON VAN LEEUWENHOEK. Arcana Naturæ. Four volumes. London. 1656.

This is by far the most important historical work on the use of the microscope. In Latin. Some very good illustrations. Very rare; found in a few libraries only.

K. C. MEZ. Mikroskopische Wasser Analyse. Berlin. 1898.

An excellent German work treating of the bacteriological investigation of drinking water and sewage waters.

GEO. NEWMAN. Bacteria. G. Putnam's Sons, New York. 1899.

Treats of bacteria in industrial processes, bacteria in public health, in nature, in soil, etc. A very valuable work, excellent for general reading.

T. M. PRUDDEN. The Story of Bacteria. G. P. Putnam's Sons, New York. 1889.

Very interesting reading on general bacteriology and on the relationship of bacteria to health and disease.

M. J. ROSENAU. The Bacteriological Impurities of Vaccine Virus. U. S. Public Health and Marine Hospital Service. Hygienic Lab. Bul., No. 12. 1903.

Of special interest to pharmacists. It should be borne in mind, however, that since the publication of this report the methods of vaccine manufacture have been modified somewhat, and the figures and results given may no longer apply.

M. J. ROSENAU. An Investigation of a Pathogenic Microbe of Rats and Mice (*B. typhi-murium* Danysz.). Washington, D. C. 1903.

This treatise is also of special interest to pharmacists, as the microbe referred to is the active ingredient of several patented rat and mouse exterminators sold under proprietary names as Azoa (Parke, Davis & Co.), Rattite, Mouratus (Pasteur Co.), etc. These exterminators are still under investigation, testing, etc., and the findings in the above report should not be considered final or conclusive.

W. G. SAVAGE. The Bacteriological Examination of Water Supplies. Philadelphia. 1906.

A valuable treatise. Contains a citation of the more valuable literature on the subject. An excellent laboratory guide for the specialist.

DR. C. STICH. Bacteriologie und Sterilization im Apothekerbetrieb. Berlin. 1904.

In German only. Contains many valuable suggestions but too incomplete and too much lacking in detail for the student.

E. R. STITT. Practical Bacteriology, Blood Work and Animal Parasitology. P. Blakiston's Son & Co., Philadelphia. 1909.

Primarily for medical students, especially those interested in the parasitology of the tropics. Complete on methods. Full details regarding blood work and use of hemacytometer.

JOHN TYNDALL. Floating Matter in the Air. London. 1881.

A very interesting popular work on the micro-organisms of the air and their relationship to fermentation and putrefaction. For general information.

NOAH WEBSTER. A Brief History of Epidemics and Pestilential Diseases. Two volumes. Hartford. 1799.

Of great historical interest, though entirely antiquated and of no scientific value.

## CHAPTER III.

## GENERAL MORPHOLOGY AND PHYSIOLOGY OF BACTERIA.

Bacterium (plural, bacteria) is a misleading term, though firmly established in general usage. It means "a small rod," the name being applied because it was believed that these minute organisms were mostly, if not all, rod-shaped. This is not the case, as will be explained later. Furthermore, the term is used in a generic sense, and again applied to the group of organisms as a whole. This causes confusion. Therefore, the generic term *Bacterium* is now abandoned and the term *Bacillus* is used to include all of the micro-organisms which are rod-shaped although generic sub-divisions are being made of this now very large group. The term "microbes" (*micros*, small, and *bios*, life) or micro-organisms would be far more suitable than the term "bacteria," as applied to the entire group of organisms included in the subject of bacteriology. Microbiology is no doubt more correctly descriptive than bacteriology, but the latter term is so firmly established in general usage that it would be unwise to urge a change at the present time.

Whereas the general morphology of microbes is apparently quite simple, the physiology and chemistry is extremely complex, and as yet not fully understood. The morphological simplicity is no doubt only apparent, and not real. Perhaps, with the greater perfection of the compound microscope, we may discover marked structural differences which thus far have escaped our notice.

### 1. Classification Of Microbes.

Microbes are the smallest of the known living organisms. It is wholly impossible to see the single individual, even the largest, with the naked eye. The rod-shaped microbes (bacilli) range from 0.5$\mu$ to 10$\mu$ in length. Some are so minute as to pass through the pores of the finest clay filters (microbes of foot and mouth disease). To study them a good compound microscope is absolutely necessary, though, as stated in the historical review (Period II), Leeuwenhoek and others observed the larger forms under the simple microscope.

The systematic position of microbes has from time to time received much attention. The great majority of biologists now unhesitatingly class them as plants, belonging to the group fungi. It cannot be denied, however, that their origin (phylogeny) is still shrouded in mystery. Some suggest that they are derived from degenerate algal forms, in common with most of the fungi, while others declare that they in all probability originated as microbes. A

few of the philosophical biologists, as Ernst Haeckel, place them in a separate group, the Monera, which is supposed to form the connecting link between plants and animals.

Without entering into lengthy discussion, we shall, in conformity with the opinion of the majority, class them as plants, belonging to the lowest of the group fungi (the fungi includes rust, smuts, cup fungi, moulds, spot fungi, toad-stools, etc.), namely, the Schizomycetes or fission fungi, so called because they multiply by fission or division. They are related to the yeasts, though somewhat lower in the scale of evolution. They are single-celled, each cell forming a complete living unit, though the several units may be variously arranged into chains or clusters or groups known as zoogolea.

The scientific grouping of microbes is as yet very unsatisfactory because so little is known of their ultimate morphology, their physiology and chemistry. Some have attempted to classify them as to form, others as to occurrence, as to action, etc. Thus, we have:

a. Micrococci or Coccaceæ.—Globular or non-elongated microbes.

b. Bacilli or Bacteriaceæ.—Cells more or less elongated. Rod-shaped microbes.

c. Spirillæ or Spirillaceæ.—Cells elongated and more or less spirally twisted. Or, we may have:

a. Bacteria of earth.

b. Bacteria of air.

c. Bacteria of water.

Or, again:

a. Chromogenic.

b. Zymogenic.

c. Pathogenic, etc.

These artificial groupings could be extended indefinitely, but such systems of classification would be as unsatisfactory as they are unscientific. The best system makes use of all of the known facts of bacteriology. Several such systems have been proposed from time to time, but the new discoveries along bacteriological lines make it necessary to change them in the course of two or three years. Migula, Fischer, Eisenberg and others have proposed general systems, and a host of investigators have submitted more limited group systems. The following classification will serve to convey some idea as to the structural characteristics of the more important groups:

### BACTERIA OR MICROBES.

(Schizomycetes or Fission Fungi.)

I. *Family* COCCACEÆ.—Micrococci. Cells globular or not elongated. Division in two or three directions of space. Spore formation rare.

1. *Micrococcus.*—Cells spherical or biscuit-shaped. Division in one direction of space. With or without flagellæ. A large genus, represented by numerous species, pathogenic and non-pathogenic, chromogenic, zymogenic, etc.

2. *Streptococcus.*—Generic limitation not clearly defined. Often merely chain forms of above, resulting from cohesion of cells dividing in one direction of space.

3. *Sarcina.*—Division in three directions of space. Cells often in fours (Tetracoccus)—as for example, the sarcina of the stomach. With or without flagellæ.

II. *Family* BACTERIACEÆ.—Bacilli. Cells more or less elongated, cylindrical, straight; some are somewhat curved or irregular in outline. With or without flagellæ. Endospore formation. Transverse septation.

1. *Bacillus.*—Variable in size and length of cell. Numerous flagellæ. Endospore formation common. A very large group, to which belong many of the most important microbes. Includes the old genus *Bacterium.*

2. *Pseudomonas.*—Said to have only polar flagellæ. Doubtful genus, by many relegated to the group bacillus.

III. *Family* SPIRILLACEÆ.—Spirillæ. Cells elongated and spirally twisted. Transverse septation. Body fixed, with polar flagellæ.

1. *Spirillum.*—Numerous polar flagellæ. Large group.

2. *Microspira.*—Few polar flagellæ. A group Spirosoma is said to be without flagellæ.

IV. *Family* SPIROCHETACEÆ.—Spirocheta. Long, single-celled, flexible, spirally twisted threads without flagellæ. One genus—*Spirocheta.* (Some authorities place these organisms in the animal kingdom with the Protozoa.)

V. *Family* MYCOBACTERIACEÆ.—Filamentous organisms, perhaps forming a connecting link between bacteria proper and the lower filamentous fungi. Cells filamentous but not enclosed in a sheath. To this family belong the groups *Mycobacterium* and *Actinomyces* (ray fungus). No flagellæ have been observed. Mostly transverse septation. Gonidial (spore) formation has been observed.

VI. *Family* CHLAMYDOBACTERIACEÆ.—Resembling above family, but the cell filaments are enclosed in a sheath. The following not very clearly defined groups are recognized: *Cladothrix*, *Crenothrix*, *Phragmidiothrix*, and *Thiothrix.*

VII. *Family* BEGGIATOACEÆ.—Beggiatoa. Family characters not clearly defined. Motile, though no flagellæ have been observed. *Beggiatoa* is the most important genus.

The uncertainty in the systematic grouping of microbes need not cause any worry, as even the leading specialists do not give the matter any con-

siderable attention, for the simple reason that their time is taken up by matters of far greater importance, namely, the determination of the rôle which the microbe plays in the life economy. Should the student ever be placed in position to justify him in attempting to identify a given microbe, he will find an extensive literature which will aid him in his efforts. Undoubtedly in time there will be a fairly simple, scientific, and complete system of classification of all known bacteria. As yet such a system does not exist.

## 2. General Morphology of Microbes.

As already stated, the morphology of microbes is simple. They consist of a single cell composed of cell-wall and cell-contents. The cell-wall con-

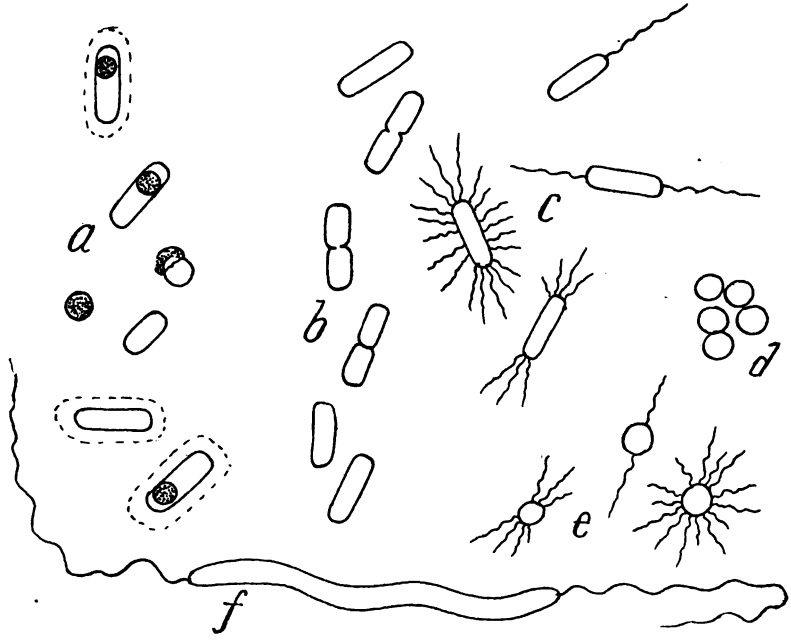


FIG. 4.—Illustrating the general morphology of microbes. *a*, showing general structure of a bacillus, endospore formation, and development of new bacillus from a spore; *b*, showing manner of transverse septation; *c*, arrangement of flagellæ, single unipolar, single bipolar; and multiple, polar and general; *d*, cocci; *e*, flagellæ of cocci; *f*, spirillum with single polar cilia.

sists of cellulose, and is very thin; stains readily with the various bacterial stains. The chief cell-contents is the cytoplasmic or protoplasmic living base commonly designated as the nucleoplasm, which is of a granular nature,

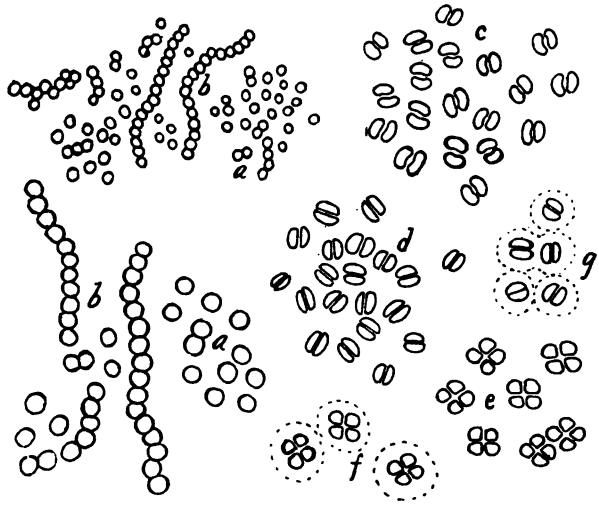


FIG. 5.—Illustrating the general morphology of Coccaceæ. *a*, *b*, micrococci (*a*) differing in size, showing chain formation or streptococci (*b*); *c*, diplococcus; *d*, diplococcus; *e*, tetracoccus; *f*, gelatinized tetracoccus; *g*, gelatinized diplococcus.

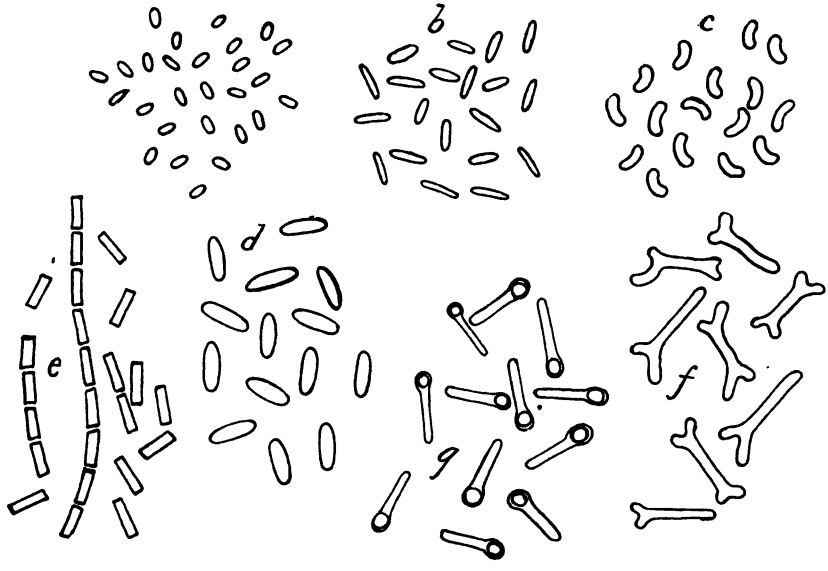


FIG. 6.—General morphology of Bacteriaceæ. *a*, *b*, *c*, *d*, bacilli differing in size and form; *c*, shows curved bacilli like those of Asiatic cholera; *e*, hay bacillus (*B. subtilis*); *f*, Y-shaped or branched bacilli, as of clover root nodules; *g*, drum-stick (Trommelschläger) bacilli, as of tetanus—form due to the enlarged endospores.

and by some is supposed to be a nucleus in a divided state. A nucleus proper does not exist, or, rather, has not been demonstrated. The cytoplasm, as a rule, stains quite readily. Distributed through the cytoplasm may be found various substances, elaborated by cytoplasmic activity. Polar granules (metachromes or Babes-Ernest granules) have been observed. Sulphur, fat, pigment, chlorophyll, etc., may be found.

The cell-walls of many species undergo a gelatinous change. This change may affect the outer layers only, or it may involve the entire thickness of the wall, forming the gelatinous substances noticeable in bacterial cultures and in other substances (stringy cultures, stringy milk, etc.). This gelatin-

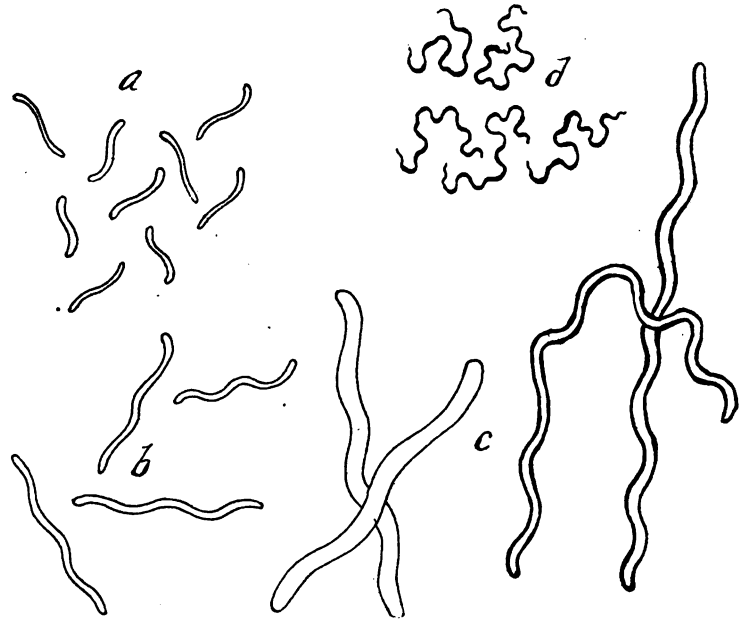


FIG. 7.—General morphology of the Spirillaceæ. *a*, S-shaped or single spiral; *b*, double spiral; *c*, multiple spirals; *d*, slender threads; *a* and *b* have fixed bodies, motion being caused by flagellæ; *c* and *d*, bodies flexible, motion not due to flagellæ.

ous substance also causes the individual organisms to cling to each other, thus causing the formation of the peculiar zooglea masses in natural as well as in artificial culture media.

The cilia or flagellæ are very delicate threads, supposed to extend from the cell-plasm, through the cell-wall, into the surrounding medium. The delicate threads are probably cytoplasmic in nature, and by their rapid vibratory motion enable the microbe to move about within liquid media. Some microbes are apparently without flagellæ, nor is it definitely determined that all motile microbes have flagellæ. Some authorities are inclined

to the belief that perhaps nearly all, if not all, micrococci and bacilli have active motion under certain conditions. This makes it clear that the attempt to group microbes into motile and non-motile must result in failure. The attempt to make generic distinctions based upon the absence or presence of few or many flagellæ, upon the existence of polar or non-polar flagellæ, etc., is also unsatisfactory. Special staining methods are necessary to demonstrate the presence or absence of flagellæ. Some investigators declare that it is almost impossible to demonstrate them ocularly. That they do exist is fully demonstrated, but it is not demonstrated to any degree of satisfaction that it is practicable to make finely drawn numerical and structural distinctions in the flagellæ of the different species of microbes.

The rate of motion of bacteria has been measured. The cholera bacillus moves at the rate of 18 cm. per hour. The typhoid bacillus is slower moving a distance of 4 mm. in one hour. The rate of motion in one and the same

Fig. 8.—Illustrating polymorphism or pleomorphism. Involution forms of the bacillus of Asiatic cholera. (*Williams.*)

species is, however, variable, being comparatively rapid at one time under certain conditions of food supply, warmth, etc., and at other times comparatively slow.

When the microbe approaches the end of the life cycle, or when the conditions for growth and septation are no longer good, spore formation is apt to take place. This spore formation is of two kinds, endospore formation and arthrospore formation. The former predominates, and occurs largely in the group bacilli, though it is also noticeable among the micrococci and the spirillæ. Endospores are usually spherical, though they may be slightly elongated, and usually occur near one end of the cell, and usually there is only one in each cell. Generally the diameter of the spore is equal to or somewhat less than the diameter of the cell-lumen. Sometimes, however, the diameter of the spore exceeds that of the cell-lumen, causing a characteristic bulging, as in the tetanus bacillus (drum-stick bacillus, *Trommelschläger Bacillus*). The spore is formed from the cytoplasm, and differs

from it in its higher refractive index and its peculiar resistance to the action of stains. As soon as spore formation is complete, the rest of the cytoplasm dies, the cell-wall disintegrates, and the spore is thus set free. Spores have a remarkable resisting power to high temperatures and other unfavorable conditions. In a dry atmosphere they may lie dormant for a long time, even several years. Boiling from one to two hours does not kill some of them (spores of hay bacillus). As soon as the spores are placed in suitable media (adequate warmth, moisture, and food supply) they develop into new individuals, which continue to septate until spore formation again takes place.

FIG. 9.—Illustrating polymorphism or pleomorphism. *a* to *d*, inclusive, represent different forms of the same organism—the Diphtheria bacillus. (See also Figs. 46–50 inclusive.)

Arthrospore formation is less common, and occurs mostly among the micrococci. The entire cell is converted into a spore, which becomes somewhat enlarged and encapsuled, in which state it is enabled to tide over certain conditions unfavorable to normal growth and septation. Arthrospore formation is not well understood as yet. It may also be that some of the phenomena described as arthrospore formations are in reality endospore formations.

The classification given above, into families and genera, and Figs. 2 to 10, inclusive, will serve to give a fairly good idea of the general structural characteristics of microbes.

### 3. General Physiology of Microbes.

Microbes, in common with living things generally, spring from pre-existing parents, take in and assimilate food, grow and multiply, and finally die. The rate of growth and of multiplication (septation or division) varies

somewhat, depending on temperature, moisture, and food supply. The average life of one individual (from septation to septation) is perhaps thirty minutes. Under favorable condition the period is much shortened. This life period of the individual cell must not be confounded with the life cycle of the individuals resulting from a single cell or parent. It is known that under uniform conditions of temperature, moisture, food supply, and the environment generally, the progenations from a single parent cell show an increasing rate of septation, a stationary period, followed by a gradual decline, ending in total cessation of all septation, and in death. These life cycles have not

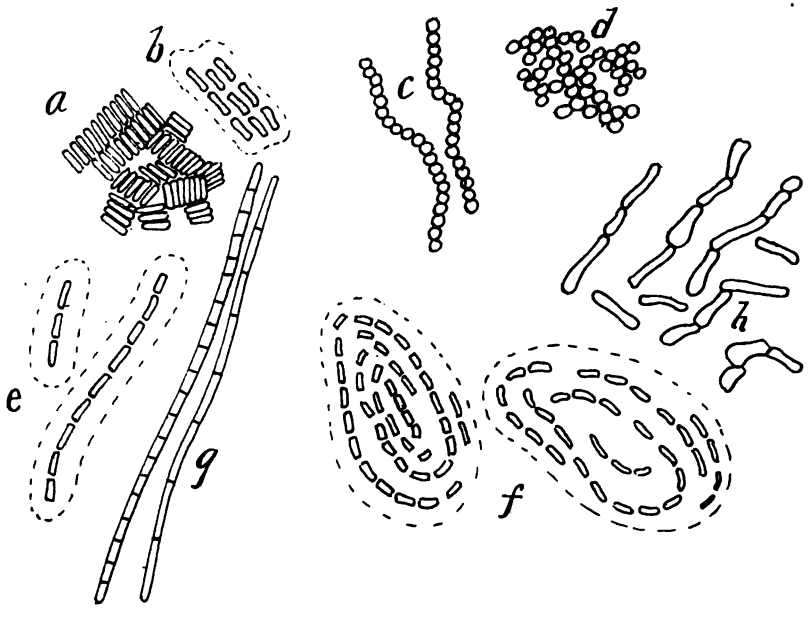


FIG. 10.—Illustrating zooglea formation. *a*, bacillar aggregates resulting from cohesion; *b*, aggregates resulting from cohesion of bacilli with gelatinized cell-walls; *c*, streptococcus formation resulting from the septation of a coccus form; *d*, cohering cocci forms; *e*, bacilli united end to end (resulting from septation), enclosed in a gelatinous coat; *f*, bacillar thread enclosed in gelatin; *g*, mycobacterial form; *h*, irregular cell forms, as *Mycoderma aceti*.

yet been carefully determined; in fact, they are but little understood. It is highly probable that the cycles of existence play a very important part in the course and development of diseases of bacterial origin.

Whereas the period from one septation to another septation is very short, the life cycle referred to is often quite long, perhaps months and, under certain conditions, lasting for years. The period of the life cycle can be modified artificially by food supply, chemicals, etc.

Investigators have succeeded in prolonging the life cycle of *Paramecium*. Normally *P. caudatum* dies out in about 175 generations; but by applying alcohol (1–5000 to 1–10,000) the cycle has been increased to 860 generations. Very dilute solutions of strychnine gave similar results. If the life cycle or vital impulse of these simple organisms can be prolonged it is probable that similar effects can be produced in higher organisms. Numerous investigators have from time to time sought after agents which might inhibit the senile changes in cells and circulatory system (arteriosclerosis) but thus far without conclusive results. It is, however, highly probable that within a comparatively short time means may be found to prolong the life of the higher animals from 10 to 20 per cent. and even more.

Microbes feed upon organic substances generally. Those which feed upon dead organic substances are said to be saprophytic; those feeding upon living substances are said to be parasitic. If they can live on dead organic substances only, they are obligatively saprophytic; if they can feed on both dead and living organic substances, they are facultatively saprophytic, or, *vice versa*, facultatively parasitic. The great majority of microbic parasites are facultatively so, as is evidenced by the fact that they can be grown in artificial culture media. Many of the microbic saprophytes will develop on living substances under certain conditions, thus showing that they are facultatively parasitic. It is no doubt true that no known microbic parasite actually feeds upon the living substances of the various hosts, since the cytoplasm is in all instances dead before it is taken up and assimilated by the microbe. It would therefore be more correct to say that parasitic microbes are biologically associated with living organisms, while the saprophytes are biologically associated with dead organic substances, and that they all feed upon and assimilate dead organic substances. In certain mutualistic symbioses (as in the root nodules of the Leguminosæ) the biological relationship of microbe and host plant is very intimate, but there is no actual interchange of living material.

All microbes require moisture and warmth (comparatively speaking) for their development, although they are enabled to withstand greater extremes of heat and cold than other organisms. The temperature of liquid air (about —270° F.) does not kill them at once, and the spores may be boiled for some time without destroying their germinating power. Cold (freezing temperature) promptly checks growth and septation, and so does dryness and excessive warmth, although life may not be destroyed. The majority of microbes develop most actively at a temperature of 25° C., a few species develop more actively at a lower temperature (20° C.), and a few others at a higher temperature (38° C.). Those which develop at a temperature ranging from 0° C. to 30° C. are said to be cold loving (psychrophile), from 10° to 45° C., mesophile, from 40° to 70° C., thermophile. Thermophile species are

found in decaying vegetable matters, whereas psychrophile species are found in cold water and cold soils.

Bacterial life processes result in the formation of many substances, some of which are of the greatest importance. It is impossible to estimate properly the enormous tasks performed by these minute organisms, nor shall we at this time make any attempt to set forth the great good and the apparent great harm done by them. We need only state that without rotting microbes soil formation would be impossible, and without soil, higher plant and animal life, as we now know them, would be impossible. Without plant food digesting microbes crop growing would be impossible. The saltpeter deposits in South America and the iron deposits of the Mesabi range of Minnesota are said to be the result of bacterial action. We make extensive practical use of microbes in medical practice, in the dairying industry, etc.

We will mention only a few substances of undoubted microbic origin. Ptomaines and toxalbumins are well-known poisons elaborated by saprophytic microbes which feed on meats and other organic substances, causing the familiar putrefactive changes. Pathogenic microbes elaborate toxins to which are due the manifestations of the disease. Acetic acid, lactic acid, and butyric acid are elaborated by *Bacillus aceticus*, *B. acidi lactici*, and *B. butyricus*, respectively. Some species liberate odoriferous substances, others gases, coloring substances, phosphorescence, etc. The phosphorescence observed on the ocean is supposed to be due to bacteria (*Bacillus phosphorescens indicus*). Phosphorescent bacteria occur in dead fish and in meat. Old cultures in animal nutrient media and in the presence of sodium salts are phosphorescent in the dark, sufficiently so, to have suggested making bacterial lamps and signal lights.

It has been suggested that certain diseases, of which the causes are at present unknown (as yellow fever, measles, whooping cough), may be due to organisms so small as to be invisible (ultra micro-organisms). It is known that the virus of yellow fever will pass through the most compact clay or porcelain filter. Attempts have been made to demonstrate the presence of ultra micro-organisms by special photomicrographic methods, aided by special illuminating devices (the ultra microscope of Siedentopf and Szigmondy) but without success. Furthermore, no one has succeeded in culturing such theoretically surmised organisms in artificial media, which would certainly render them visible *en masse*. It may, however, be possible that some ultra-organisms are obligative parasites hence will not develop in artificial media.

The biological (symbiotic) relationship of different species of bacteria to each other and to their host are, in many instances at least, not well understood. For example, it is not clear what biological relationship the different species of bacteria in a mixed infection bear to each other. In the case of the

root nodule organisms of the Leguminosæ it is known that there is a mutually beneficial (mutualistic symbiosis, mutualism) relationship between microbe and host but it is not obligatively so, since the symbionts can exist independently of each other. In most diseases due to microbic invasion there is one species of bacterium which acts as the primary cause. It is known that tuberculosis, especially the pneumonic form, usually shows a mixed infection, and it is probable that the associated organisms as bacteria and higher fungi act as predisposing causes, preparing the tissues so as to yield more readily to the invasion of the primary cause, the *Bacillus tuberculosis*. Such an association may be designated compound symbiosis, in which the relationship of the invading organisms (secondary and primary) is mutualistic and the relationship of these to the host is antagonistic. It is known that certain microbic diseases predispose to other microbic invasions, thus we may say that these organisms are mutualistically disposed toward each other.

Since it is possible to cultivate most disease germs in and upon artificial culture media (hence dead organic substances) it is evident that they are only facultatively parasitic.

In many instances the biological association of bacteria and higher plants and animals is loosely mutualistic, as the bacteria upon roots and rootlets of all plants and the bacteria lining the intestinal tract of animals. The hay bacillus (*Bacillus subtilis*) is a constant associate with the Gramineæ and serves an important function, assimilating or binding for the use of the host plant, the free nitrogen of the air. Certain soil organisms (*Bacillus megatherium, B. ellenbachiensis, B. mesentericus, B. pyocyaneus, B. prodigiosus*, the Azotobacter group, *Clostridium pastorianum*, certain moulds as *Aspergillus niger* and *Penicillium glaucum*) are capable of assimilating the free nitrogen of the air thus enriching the soil for the benefit of higher plants.

## CHAPTER IV.

## RANGE AND DISTRIBUTION OF MICROBES.

Microbes are omnipresent over the surface of the earth. In number and in bulk they exceed all other organisms (plants and animals) put together. They form a large precentage of the bulk of the soil. They occur in the air, in water, in snow, in hail, in raindrops, in and upon plants, in and upon animals. All substances with which we come in contact are likely to hold microbes. Our clothing teems with them. They are in the air we breath, in the food we eat, and in the liquids we drink. The floating dust particles of the air carry microbes; the particles of organic matter in water harbor microbes; they are found on wood, on cloth, on paper, on metal, glass, and rock surfaces, in fact on all exposed surfaces. The hands, the hair, the entire body surface of man and of the lower animals contain or hold microbes. They line all mucous membranes. The mouth cavity is a veritable bacteriological laboratory. The entire intestinal tract teems with millions upon millions of these minute beings.

Each animal and each plant has a microbic flora peculiar to itself. Each portion of the plant or animal, again, has distinctive bacterial groups. The microbic flora of the intestinal tract of the dog is different from that of the pig, or cat, or fowl, or man. Certain species predominate in the mouth cavity, others in the stomach, still others in the small intestine, in large intestine, etc.

Microbes are found on the highest mountain peaks and in the deepest valleys. It is, however, true that the higher atmospheric strata contain fewer microbes than the lower strata. The deeper layers of soil contain fewer microbes than the upper. The atmosphere of the country contains fewer microbes than that of the cities and towns. Since sunlight and absence of moisture are natural enemies of microbes, we may expect to find microbes more abundant in dark, damp, and moist places and areas. Microbes are always more abundant in cellars, basements, dark hall-ways, and alleys than they are in attics, sunlit living rooms, and along broad boulevards and highways.

Good drinking water, whether from hydrant, spring, or well, contains only a comparatively few microbes, from fifty to one hundred per c.c., or even less. Stagnant, foul water teems with microbes, besides other organisms, such as protozoa. So-called pure milk contains comparatively more microbes than pure water. The average good milk contains as many as 30,000 microbes

per c.c. Filthy milk may contain millions of microbes per c.c. From 100,000 to 3,000,000 microbes per c.c. is not uncommon in some milk which careless dairymen declare to be "good." Soups, broths, etc., boiled squash, potatoes, meats, and cooked organic substances generally, if allowed to stand for a day or two, contain many living microbes. In the course of two or three days, if the weather is warm, these substances teem with microbes and are rendered wholly unfit for food because of the predominating rotting microbes which develop the highly poisonous ptomaines.

Microbes do not live and multiply in aseptic and antiseptic substances, such as strong solutions of acids, of alkalies, of salts, etc. Used and dirty cups, drinking vessels, milk bottles, dishes, cooking utensils, knives, spoons and forks, hold numerous microbes. The public drinking cup has been the source of numerous disease infections. Disease is carried by the tools of the careless dentist and by the clothing, the apparatus and the clinical thermometer of the indifferent and careless physician. The hand-shaking and kissing habits spread disease. These facts are generally known and indicate the wide dissemination of the different kinds of microbes.

From the foregoing it becomes clear that microbes are present almost everywhere, and that it is impossible to escape them. It is the aim of the science of bacteriology to distinguish between good and bad microbes, between those which are desirable and those which are undesirable, between useful and harmful microbes. It is not the aim of the science of bacteriology to destroy them all, or to devise ways and means to escape from all of them. In fact, we owe our very existence to these very minute organisms, as has already been explained.

Under certain conditions bacteria multiply very rapidly. Such substances as meat, milk, and organic foods of all kinds, if exposed to moisture, warmth and removed from sunlight, soon swarm with microbes. Certain non-pathogenic microbes, as the root nodule bacteria (of the Leguminosæ), multiply very rapidly within the tissue cells. Others multiply upon the exterior of roots and of root hairs, where they no doubt serve a useful purpose to the plant. In bacterial diseases of plants and animals the microbes multiply very rapidly and form large aggregates, as a rule. To pathological conditions accompanied by extensive and general bacterial or microbic invasion, we apply the term bacteremia. In some diseases the microbic invasion remains localized and yet there are pronounced general or systemic effects, due to the absorption, into the system, of the toxins liberated by the microbes. To such conditions we apply the term toxemia. Toxemia may, however, also occur in bacteremia.

Microbes do not multiply in the air itself, rather upon the organic dust particles present, provided warmth and moisture are adequate.

Since microbes multiply rapidly, perhaps one septation in from twenty to

thirty minutes, it is evident that the rate of numerical increase, under favorable conditions, is very great. Allowing thirty minutes for each septation, there would be a colony of 2,097,152 microbes in ten hours, developed from a single cell, or about 75,000,000,000,000 cells in twenty-four hours. However, under natural conditions septation never proceeds in such uniform ratio. All manner of checks to septation come into play sooner or later which may finally bring about complete cessation of septation and sporulation.

## CHAPTER V.

## BACTERIOLOGICAL TECHNIC.

As may readily be supposed, the minuteness and wide distribution of microbes call for special methods of study and examination. Even the largest forms are far below the ken of unaided vision. Their general dissemination through organic substances calls for special methods for the separation and isolation of individuals or of single bacterial cells. The difficulties of technic are further increased by the resistance of spores to various agents and substances which are readily fatal to higher organisms. The methods of examination are also greatly complicated by the marked polymorphism of many species.

Bacteriological technic comprises the use of glassware, compound microscope, and other apparatus, a thorough knowledge of sterilization and disinfection, the preparation and use of culture media, the making of micro-

*a*

FIG. 11.—*a*, Nest of beakers and reagent bottles. The smaller and medium size beakers are more desirable for bacteriological work. The reagent bottles are for Canada balsam, stains, clearing fluid, etc.

bic cultures, and the study of cultures. Methods vary greatly. The following represents a brief summary of general methods which are noted for simplicity and which have proven very satisfactory after years of testing.

### 1. Cleaning the Glassware.

All glassware, such as test-tubes, flasks, beakers, Petri dishes, pipettes, shells, bottles, etc., which is to be used in bacteriological work must be *clean;* that is, free from all extraneous organic as well as inorganic matter. To accomplish this, it is necessary to use an abundance of pure water, hot as well as cold, aided by sand, paper shreds, brushes, towels, alcohol, acids, soap, sodic and potassic hydroxides, and whatever else may be necessary. Boil, wash, rinse, and wipe within and without repeatedly until it looks, and

is, absolutely clean. The following solution will be found useful as a cleansing agent for old as well as new glassware:

| | |
|---|---|
| Potassium Dichromate, | 6 parts. |
| Sulphuric Acid, | 30 parts. |
| Water, | 40 parts. |

Of course, the sulphuric acid must be added little by little with constant stirring, in order to avoid excessive heat development. Soak the glassware

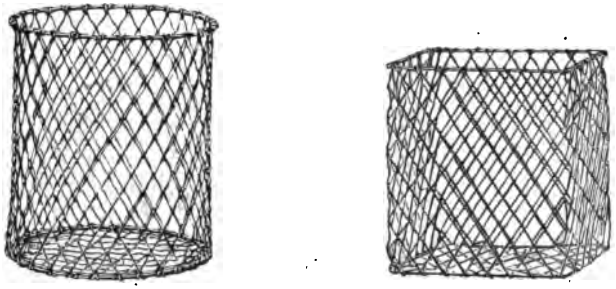

FIG. 12.—Wire baskets for holding test-tubes. Cylindrical form and square form. Each basket holds about fifty test-tubes. The wire is galvanized to prevent rusting. The round wire baskets should be used.

in this solution for some time, several hours or more, and rinse, wash, drain, and wipe thoroughly afterward. The sole object to be attained is *cleanliness* in the true sense of the word. The glassware must be clean bacteriologically and chemically; that is, it must be free from microbes and chemical substances.

## 2. Plugging Containers with Cotton.

After the thorough cleansing above outlined, the test-tubes and flasks are plugged with a good quality of non-absorbent commercial cotton. The dry cotton plug forms a most efficient germ filter. All microbes are caught and held in the meshes of the cotton, and yet the air is permitted to pass through into the tube or flask.

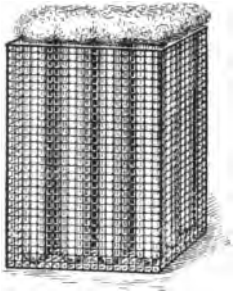

Fig. 13.—Wire basket filled with test-tubes plugged with cotton. A little cotton should be placed in the bottom of the basket to lessen the danger of breaking the test-tubes. (*Williams.*)

Open a roll of cotton, find the free end, and lay it out on the work table. Take the test-tube in the left hand; remove a goodly tuft of cotton with right hand, using thumb and first and second fingers. Place this over the mouth of the tube or flask, and push it down to a distance of 1/2 to 3/4 inch by means of a solid glass rod rounded (by heat) at the ends. The rod must not be too

thick, as it will then not permit enough cotton to enter the opening n or yet too thin, as it will then be forced through the cotton. The plug must not be too tight, as that would interfere with subsequent manipulations nor, yet too loose, for obvious reasons. Enough cotton should project above the opening to permit of ready grasping between the fingers in the later operations.

Plugging may also be done with fingers alone, but this is tedious and non-professional. A far better method is to use a pair of fairly large blunt-

FIG. 14.—A hot air sterilizer. These sterilizers are double-walled, on stand, with perforations at top for thermometers. Ordinary baking ovens which can be secured from hardware dealers will serve the purpose.

pointed pincers. Remove the cotton from the roll by means of the pincers and insert it into the test-tube with the pincers.

Whatever method is used, remove the amount of cotton required to plug one tube or flask at one time. Do not attempt to plug with several small pieces. If an excess of cotton projects above the opening, pluck it away with the fingers; do not cut it away with scissors. Plug the tubes as uniformly as possible.

### 3. Filling Test-tubes with Culture Media.

The rule is to pour the culture media hot, although this is not absolutely essential. For example, if the media are liquid in the cool or cold state, as bouillon, serum, milk, etc., they may be poured cold. A good rule is to pour a desired amount of the media just as soon as they are prepared, whether they are still hot or merely warm or cold. Of course, gelatin and agar media must be poured hot or must be liquefied before they can be poured.

Fill a small to medium-sized beaker about two-thirds full of the culture medium. Grasp a plugged tube near the upper end, holding it between thumb and first two fingers of the left hand. Remove the cotton plug by means of the first and second, second and third, or third and fourth fingers of the right hand, grasping the free portion of the plug with the back of the fingers toward the cotton. Holding the tube slightly inclined on a level with the mouth, take beaker with medium in right hand (at the same time holding the cotton plug as described), see that the beak rests lightly upon and projects slightly over the edge of the tube, and pour, at the same time shifting the eyes to the lower end of the tube to watch the filling process. Fill tubes one-third full. Set down the beaker and replace the cotton plug. Place the filled tubes in special wicker baskets, with a little cotton at the bottom to prevent breaking. Some practice is necessary in order to pour so that none of the liquid comes in contact with the upper third of the tube. This must be avoided, in order to prevent the cotton plug from sticking. Tubes may also be filled from funnel with rubber hose, stop-cock, and glass nib attachment. Occasionally it is desirable to place exact amounts of culture media in the tubes, in which case a graduate, a burette, a pipette, or other convenient measuring device may be used.

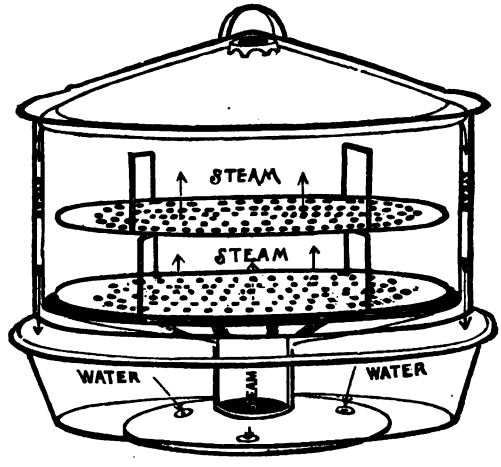


FIG. 15.—Diagrammatic sectional view of Arnold steam sterilizer illustrating the principle of steam formation, circulation and condensation.

### 4. Sterilization of Culture Media.

All culture media in tubes as above set forth, and the portions remaining after the desired number of tubes are filled, must be considered as being contaminated with living microbes and their spores. These microbes and spores are killed by the sterilizing process. For all ordinary purposes the

discontinuous or fractional method answers the purpose admirably. Place the test-tubes, flasks, and other cotton-plugged containers with culture media, in a steam sterilizer (Arnold steam sterilizer, either board of health or cylindrical form; or kitchen vegetable cooker or steamer). The test-tubes are placed in wire baskets (rectangular or cylindrical). These several containers with culture media are exposed to live steam for about thirty minutes, whereupon the flame is turned out, and if convenient the containers are allowed to remain in the sterilizer. Caution must be observed to guard against condensed steam running into the several containers. The better way is to remove the containers and place them in an incubator kept at a temperature of 20° C. In twenty-four hours, or thereabouts, steam is again applied for thirty minutes. This is repeated a third time on the second day after the first sterilization. The first sterilization presumably kills most of the vegetative cells. During the first interval of twenty-four hours most of the spores present develop into vegetative cells, which are killed at the second sterilization. Should any survive the second steaming, they are sure to be killed during the third sterilization. During this time the cotton plugs have not been removed. The media thus fractionally or discontinuously sterilized are now ready for use in making microbic cultures, or they may be set aside for an indefinite period of time.

FIG. 16.—Autoclavé for using steam under pressure for purposes of sterilization.

It is, of course, evident that in the above process of sterilization the temperature does not exceed 100° C., and it may be less in certain portions of the sterilizer, steamer, or cooker, say, 95° to 97° C. In large or well-equipped bacteriological laboratories certain kinds of sterilizations are done by steam under pressure. The apparatus used for this purpose is known as autoclave. It consists of a strong steam cylinder with a screwed-down top safety valve, steam gauge, and thermometer. The articles (media, etc.) to be sterilized are placed inside, the top is securely fastened down, steam is generated until the thermometer registers, say, 120° C. The temperature is kept up to that degree for about five to ten minutes, which is sufficient to destroy all life, including spores. For ordinary purposes the autoclave is not essential. In fact, its use is rather limited. Blood serum, gelatin media, and all media containing carbohydrates, undergo certain chemical changes

when the temperature is raised above 100° C., or even if kept at 100° C. for a long time or for a short time, if oft repeated. The autoclave is convenient for sterilizing discarded cultures, test-tubes, and glassware generally, and such media as beef broth and agar.

In many instances it is desirable to sterilize at a temperature lower than 100° C. Albumen and blood serum, for instance, will coagulate at that temperature. Again, it is desired to kill the microbes without destroying the toxins which they form, as in the manufacture of bacterial vaccines. In the sterilization (pasteurization) of milk, a lower temperature is employed. In the sterilization of these and other substances the temperature ranges from 50° C. to 85° C. The discontinuous method is employed, differing from the method already described in that the period of exposure is much prolonged, about one hour. The number of daily exposures ranges from one to six. For example, milk exposed to a temperature of 60° to 70° C. for one hour is considered sufficiently sterilized, whereas blood serum is subjected to hourly exposures of a temperature of 60° C. for six successive days before it is pronounced completely sterilized.

## 5. Preparation of Culture Media.

The pharmacist should give especial attention to the preparation of bacterial culture media, as in this he may be of service to the physician. The busy general practitioner who is not eqipped with a suitable bacteriological laboratory, or who does not have time to prepare culture media, would no doubt consider it a very decided advantage should the pharmacist offer to assist him. This will be more fully set forth in the last chapter.

In brief, it may be stated that microbes feed upon the same substances that we feed upon. In the presence of adequate warmth and moisture they attack all organic substances. This being the case, it may readily be assumed that there are many substances or media which can be used as food for bacteria. Such is the case, and the number of media which have been used is legion. Almost any organic substance may be used, provided it is not aseptic or antiseptic in its properties.

Culture media are liquid or solid, simple or compound. In the case of liquid or liquefiable solid media, the following physical properties are desired, in so far as it is possible to attain them:

*a.* Culture media should be perfectly clear. There should be no sediment, no opacity or flocculent suspension, and no floating matter. In the case of broths, extracts generally, gelatin media, and blood serum, these requirements are easily attained. Perfectly clear agar is difficult to obtain. Milk is normally opaque.

*b.* Media should be neutral or very slightly alkaline to litmus, which is

equivalent to a slightly acid reaction to phenolphthalein, at a temperature of about 20° C. Most microbes develop best in media of such reaction.

*c.* They must be free from living microbes and their spores, and from

FIG. 17.—Arnold Steam Sterilizer. Boston Board of Health Form. This sterilizer is square, and constructed with a side-door all in accordance with the recommendation of the Boston Board of Health. Its large size makes it well suited to the requirements of Board of Health laboratories, and it has been found to be very serviceable and convenient. It is made of copper throughout, following the same principles as employed in the construction of the other sterilizers.

FIG. 18.—Arnold Steam and Hot-Air Sterilizer for Surgical Instruments. This sterilizer is a combination and portable sterilizer, so designed that instruments may be both sterilized and then dried by hot air, if desired. About 100° C. can be attained with the hot air by simply turning the valve shown in the illustration, which turns the steam as it escapes from the chamber into the base.

other organism. This requirement is attained by sterilization as already described. Culture media contaminated with living organisms are not usable in bacteriological work.

The essential requirements given under *a*, *b*, and *c* are obtained by filtration, neutralization, and sterilization, as will be more fully explained. Non-liquefiable solid media, as potato, bread, squash, etc., must be clean, free from living microbes and other organisms, and there should be a comparatively smooth exposed inoculating surface. These requirements are attained by washing and otherwise cleansing, disinfecting, rinsing, and heat sterilization (dry heat, steam or hot-water bath).

The following are the more important media:

A. *Nutrient Bouillon.*—

| | |
|---|---|
| Beef Extract (Armour's, Liebig's, etc.), | 3 gm. |
| Peptone, | 10 gm. |
| Salt, | 5 gm. |
| Distilled Water, | 1000 c.c. |

Mix ingredients and boil for a few minutes. Filter through filter paper. This bouillon may be modified by adding glycerin (6 per cent.) and sugars, as dextrose, saccharose, or lactose (1 per cent.).

B. *Loeffler's Blood Serum.*—Very largely used in making diagnostic diphtheria bacillus cultures. In many cities this medium, with sterilized cotton swabs, in sterilized test-tubes, is furnished free to physicians by the board of health. In cities and towns where this is not done, the pharmacist should be prepared to furnish the materials to the physicians. The medium consists of

| | |
|---|---|
| Bouillon with 1 per cent. Glucose, | 1 part. |
| Blood Serum, | 3 parts. |

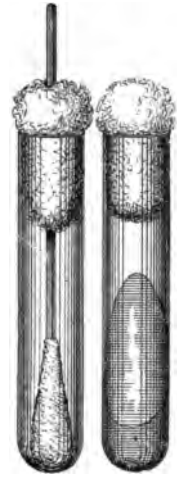

FIG. 19.—Culture tube and swab tube used by physicians in the diagnosis of diphtheria. The swab tube should be long enough to have the entire length of swab inside, not projecting as shown in the figure. (*Williams.*)

The bouillon is prepared as above described, with 1 per cent. of glucose added. The blood serum can be obtained from calf, sheep, ox, or cow, through the butcher or at the abattoir. Collect the blood in a clean, sterile jar or flask, closed with cotton plug. Place on ice for twenty-four to forty-eight hours, during which time coagulation has taken place; the serum may then be siphoned off. The proper sterilization of Loeffler's serum requires care. After the bouillon and serum are mixed, pour into test-tubes and coagulate in a Koch-serum coagulator at a temperature of 80° C. Any form of sterilizer may, however, be used. The essentials are that the temperature should be raised very gradually and must be kept below the boiling-point, and the tubes should be slanted at a degree which will bring the medium close to the cotton plug, making what are commonly called tube slants. After the medium is coagulated in the tubes it is sterilized frac-

tionally on three successive days (one hour each day) at a temperature of 80° C. These tube slants are now ready for the physician.

To prevent evaporation of the medium in the test-tubes, cover the cotton plug and upper end of tube with tin foil fastened with thread, and dip into melted paraffin several times. Tubes thus sealed can be kept for a year or more without any considerable shrinking of the medium. Dip the tin foil in a 1:2000 corrosive sublimate solution before capping on tubes.

A simpler way is to use rubber caps which are especially made to fit over the end of the test-tube and the cotton plug. These rubber caps must be sterilized before applying them, for which purpose the 1–2000 corrosive sublimate solution will be found satisfactory. Rubber stoppers may also be used but they are more expensive and inferior to the rubber cap or the tin foil with coat of paraffin.

C. *Liquid Blood Serum.*—Obtained as for Loeffler's serum. Sterilize fractionally at a temperature of from 56° to 58° C. for one hour on each of six days. The serum will be liquid and clear.

D. *Milk.*—Secure fresh milk directly from cow, or, if in cities, demand certified milk. Keep on ice, in a covered jar, for twenty-four hours. Siphon off the middle portion, rejecting cream and sediment. Sterilize like Loeffler's blood serum. Litmus milk is prepared by adding 1 per cent. of azolitmin before sterilizing. This indicator will show whether or not acids are formed by the microbes which may be cultivated in the milk. Only pure milk will answer the purpose. Milk to which preservatives (formaldehyd, salicylic acid, borax, boric acid) have been added must not be used.

E. *Peptone Solution.*—The medium is employed to test for the development of indol by certain bacteria. It consists of

| | |
|---|---|
| Peptone, | 10 gm. |
| Salt, | 5 gm. |
| Distilled Water, | 1000 c.c. |

Boil, filter, and sterilize as for bouillon. The bacteriological indol test is of great importance in medical practice, and the chances are that physicians will require this medium. However, sugar-free beef broth is also used for this test; in fact, it is generally preferred. Beef contains a small amount of muscle sugar, which must first be removed.

F. *Sugar-free Bouillon.*—Grind the fat-free beef through a meat grinder; add water, and inoculate at once with a pure culture of *Bacillus coli communis*, and allow to incubate for twelve to fifteen hours at 38° C., then boil, filter, add peptone and salt, and prepare like bouillon; or, inoculate nutrient bouillon with the colon bacillus and prepare as above. However, before using the medium it should be tested for indol, as it has been proved that *B. coli communis* may form indol in beef extract. The indol test in bacterial cultures is made by adding two drops of concentrated sulphuric acid and one

drop of a 0.01 per cent. sodium nitrite solution to a four-day peptone-broth culture. If a pink color appears at the end of one-half hour it indicates the presence of indol.

G. *Beef Broth.*—This medium is now not as extensively used as formerly. It is more difficult to prepare, and shows no advantages over the bouillon already described.

| | |
|---|---|
| Ground or Chopped Lean Beef. | 500 gm. |
| Peptone, | 10 gm. |
| Salt, | 5 gm. |
| Distilled Water, | 1000 c.c. |

Add the water to the minced meat, shake frequently, and keep on ice for twenty-four hours, then strain forcibly through cloth, or press out in a hand press. Add the salt to the liquid, boil, make up to 1000 c.c., and add the peptone. Neutralize, filter, and sterilize. It will be apparent that the cold water meat infusion contains merely the meat salts, meat sugar, and acids, and a certain proportion of the albumens. The albumens are coagulated and removed in the filtering process, so that nothing remains of the meat but the salts, acids, and the trace of muscle sugar. Nearly the whole of the meat proper is wasted. It

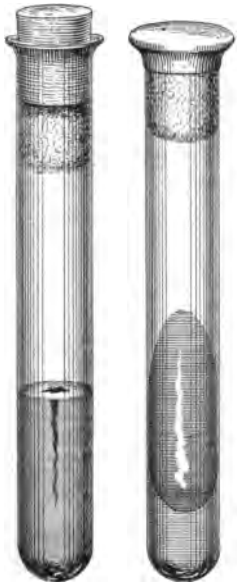

FIG. 20.

FIG. 21.

FIG. 20.—Test-tube cultures. *a*, Stab culture. This tube is closed with a rubber stopper to prevent drying of medium; *b*, streak or smear culture on slant, tube closed with rubber cap. (*Williams.*)

FIG. 21.—The ordinary rice cooker. A most valuable apparatus in preparing culture media and for sterilizing test-tubes and other objects.

is apparent, therefore, that the meat extract bouillon answers all the purposes of the beef broth.

H. *Gelatin Medium.*—

| | |
|---|---|
| Beef Extract, | 3 gm. |
| Gelatin, | 100 gm. |
| Salt, | 5 gm. |
| Peptone, | 10 gm. |
| Distilled Water, | 1000 c.c |

Mix ingredients in a rice cooker and boil for one-half hour, stirring frequently; neutralize and filter. This forms a very efficient culture medium for most bacteria, and is clear and remains solid at ordinary temperatures. It must be borne in mind, however, that frequent or prolonged heating tends to liquefy gelatin permanently.

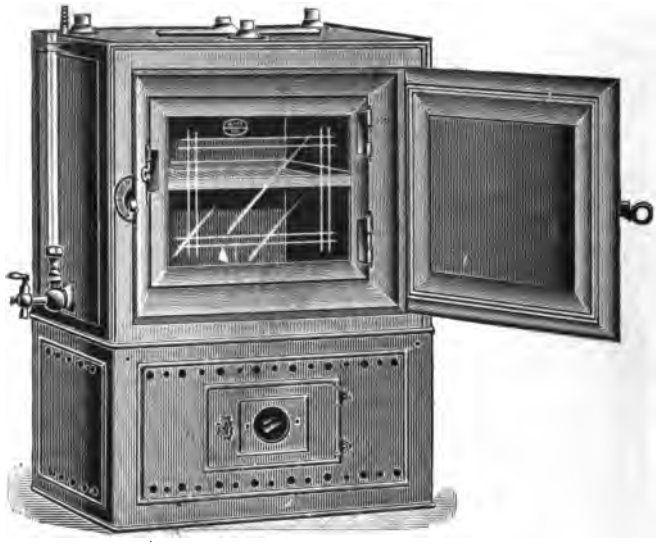

FIG. 22.—This is a copper double-walled incubator covered with non-conducting material and provided with a water gauge, tubulations for thermometer and thermostat, a ventilating strip, enclosed base and inner glass door. The incubating chamber is 24 cm. high, 30 cm. wide and 24 cm. deep.

I. *Agar Medium.*—Agar is a seaweed found on the Japanese coast. It forms an important article of diet among the Japanese and Chinese. The medium consists of

| | |
|---|---|
| Beef Extract, | 3 gm. |
| Agar, | 15 gm. |
| Salt, | 5 gm. |
| Peptone, | 10 gm. |
| Distilled Water, | 1000 c.c. |

Prepare like the gelatin medium. Agar is difficult to filter, and the medium is never quite clear. The agar medium liquefies at a higher temperature than gelatin, and does not tend to remain liquid, no matter how often or how long it may be heated.

J. *Agar-gelatin Medium.*—This has the advantage of both media, and is now much used in general bacteriological work.

| | |
|---|---|
| Agar, | 8 gm. |
| Gelatin, | 40 gm. |
| Salt, | 5 gm. |
| Peptone, | 10 gm. |
| Distilled Water, | 1000 c.c. |

Mix, boil in rice cooker, stir; neutralize, filter, and sterilize as for other media.

The above includes the more important culture media used in bacteriological work. Others can be prepared as occasion requires. It is not neces-

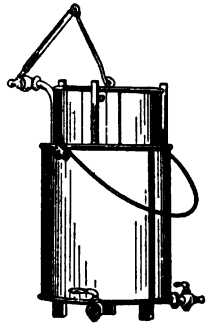

FIG. 23.

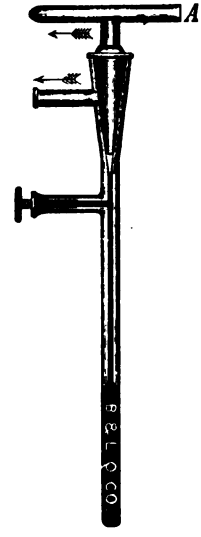


FIG. 24.

FIG. 23.—Murrill's Gas Pressure Regulator. This apparatus in its most improved form is to be used in connection with a thermostat for the maintenance of a constant emperature. The use of this regulator relieves the thermostat of the necessity of caring for the wide viriation which is apt to occur in the gas pressure, and with it the temperature may be held constant to within 0.1° C.

FIG. 24.—Reichert thermo-regulator or thermostat used with incubator and other apparatus requiring a uniform degree of temperature. May be used in conjunction with the gas pressure regulator.

sary to make up the full amounts indicated if it is evident that smaller quantities will suffice. The student should prepare all of the media in small amounts (one-quarter the quantities given) several times, in order to get the necessary experience and practice.

### 6. General Directions for the Preparation of Culture Media.

Book information alone is not sufficient. Experience must be added.

Also, brief, concise explanations are far more valuable than lengthy descriptions of unessential details. Those possessed of good judgment do not require lengthy explanations, and lengthy explanations would certainly be wasted on those who lack good judgment. This does not imply, however, that it is unnecessary to adhere strictly to established methods. The novice must follow closely the methods formulated by those who have devoted many years to some one particular mode of procedure, as it is wholly unlikely that he can improve upon them. Furthermore, when a physician calls for Loeffler's blood serum, for example, he wishes to be assured that the medium has been prepared according to the standard method. Any substitution or deviation, no matter how slight, may bring about wholly negative or erroneous results and conclusions. With this in mind the following suggestions are added:

A. *Selection of Ingredients.*—Great care must be observed in the selection of the ingredients used in the preparation of culture media. Meats used must be from healthy animals, and there must be absolute certainty that no preservative has been added. Buy the meat personally from the nearest reliable butcher who keeps fresh meats only. Remove as much of the fat as possible. The so-called round steak of beef is usually employed.

Use only the best gelatin; the so-called best French gelatin is usually employed, although much of the "French gelatin" comes from Berlin, Chicago, Omaha, or other places equally remote from France. Do not attempt to use old friable gelatin.

The milk requirements have already been referred to. The milk must be fresh, placed on ice at once, and sterilized within twenty-four hours after it is taken from the cow. If the milk is obtained from an unknown dealer, test it for the presence of added water, preservatives, and other foreign matter.

Agar does not deteriorate readily, and may be kept in good condition for a long time. Other highly gelatinous seaweeds may be used, although this is not permissible in the preparation of any of the standard culture media.

Serum, egg albumen, peptone, various indicators, etc., must be pure. Too much caution cannot be observed in this regard. Secure the blood for serum personally wherever possible, from healthy animals. Use egg albumen from fresh eggs, not from cold-storage eggs. Peptone and other chemicals should be secured from reliable dealers.

B. *Suggestions on the Preparation of Culture Media.*—First of all, some experience is necessary before a neat article can be prepared. Do not expect to prepare a medium which meets all of the requirements the very first time. In preparing gelatin media, remember that these are injured by excessive heating, and in preparing agar media, remember that they are very difficult

to filter. Both must be filtered hot, using hot-water funnels; or the ordinary filtering device can be used by keeping the unfiltered portion hot and pouring into the funnel from time to time. Cover funnel with filter paper to keep out dust, and keep in the heat as much as possible. In so far as possible filter all media through filter paper (one thickness, properly folded), but it is practically impossible (for reasons of time) to pass agar through filter paper. This medium is usually filtered through cotton upon which a neatly folded and perforated sheet of filter paper has been placed. Puncture the filter paper several times with a small knife blade. Filtering through cotton is quick, but the media are much less clear than when filtered through filter paper. The filtering process may also be hastened by means of pressure (suction); connect funnel with aspirator bottle and pump, but see to it that the connections with the hydrant are properly made and that the flow is properly regulated, in order to guard against any back pressure, which may cause the receiver to fill with hydrant water. This accident is best avoided by interpolating a flask or bottle. Agar may also be clarified by precipitation. Pour the hot agar into an ordinary percolator used by pharmacists. The dirt particles and other impurities will gradually settle to the bottom. When cool, take out the solid medium and cut away the lower portion cantaining the sediment.

C. *Neutralization of Culture Media.*—As already stated, most bacteria grow best in neutral or very slightly alkaline (to litmus) media, and since most media are quite decidedly acid in reaction, it is desirable to alkalinize. This is done by means of normal sodium hydroxide solution. In order to understand the method of procedure clearly, it is necessary to make certain explanations.

A normal (N/1) solution of any substance contains as many grams per liter of the substance as there are units in its molecular weight, if the substance contains one atom of replaceable hydrogen. If it contains two atoms of replaceable hydrogen, the number of grams used equals the molecular weight divided by two, and so on. According to this, a normal solution of sodium hydroxide contains 40 gm. of sodium hydroxide in a liter. Exact normal solutions are, however, not prepared by weight. Crystallized oxalic acid is used as the basis for making normal solutions. This acid has a molecular weight (including a molecule of water of crystallization) of 126, and, since it is dibasic, 63 gm. per liter are taken. Any normal acid solution will exactly neutralize an equal volume of normal alkaline solution. To make a normal sodium hydroxide solution, add about 41 gm. of pure caustic soda to one liter of distilled water. Determine the amount of this solution required to just neutralize 1 c.c. of normal oxalic acid solution. This volume contains the quantity of sodium hydroxide which should be present in 1 c.c. of normal solution, and from this we may

calculate the volume of distilled water to be added in order that 1 c.c. of sodium hydroxide solution will neutralize 1 c.c. of normal oxalic acid solution. Having a normal solution of sodium hydroxide, it is now possible to prepare a normal solution of hydrochloric acid, etc. A tenth- (N/10), twentieth- (N/20), fiftieth- (N/50) normal solution is a normal solution diluted ten, twenty, and fifty times.

An acid reaction is indicated by +, and an alkaline by −. The degree of acidity of any culture medium in preparation may be indicated by the amount of normal sodium hydroxide solution required to render it neutral to phenolphthalein. Neutralization by titration is done as follows: Place 5 c.c. of the medium to be neutralized in a dish, add 45 c.c. of distilled water, stir, and bring to a boil. Add 1 c.c. of phenolphthalein solution (0.5 per cent. of phenolphthalein in 50 per cent. alcohol). Add enough of twentieth-normal sodium hydroxide solution (in a burette), with constant stirring, to give a faint but distinct pink color. Read the amount of twentieth-normal sodium hydroxide necessary to neutralize the 5 c.c. of medium, and from this calculate the amount of normal sodium hydroxide solution necessary to neutralize the entire quantity of culture medium. Now boil the medium, and again titrate, when it will be found that there is a slight acid reaction. A third titration is rarely necessary.

Another method is to take 10 c.c. of the culture medium, add a few drops of the phenolphthalein solution. From a burette add, drop by drop, with constant stirring, a normal sodium hydroxide solution (0.4 per cent.) until a faint pink color appears, which indicates the beginning of the alkaline reaction. Repeat this with two more samples. Note the amount of sodium hydroxide solution required in each case, and take the average and calculate the amount required for the entire quantity of medium. If, for example, the average was 1 c.c. for each 10 c.c. of medium, then 1000 c.c. of bouillon would require 100 c.c. of the sodium hydroxide solution; a concentrated solution being used, in order to avoid the dilution of the medium with the water of the caustic-soda solution. Flocculency of the medium usually indicates excessive alkalinity.

The old, crude, rough-and-ready method is to add, from a beaker, drop by drop, a tenth-normal sodium hydroxide solution, with constant stirring, until red litmus paper just begins to turn blue. In practice it is found that when a culture medium is neutral or slightly alkaline to litmus it is still acid to phenolphthalein. In fact, it is claimed that most bacteria develop best in a medium having a reaction indicated by +1 or +0.5; that is, it is sufficiently acid to phenolphthalein to require 1 per cent. or 0.5 per cent. of normal sodium hydroxide solution to render it neutral to phenolphthalein.

D. *Suggestions on the Preparation of Culture Media for Physicians.*—First of all, the pharmacist must have the necessary laboratory equipment and

necessary skill and experience to prepare culture media. He should explain to a few representative physicians that he is ready to prepare such media as the busy physician may require. The physicians will in all probability indicate what media are likely to be needed in the course of their practice. Allow yourself to be guided by these several suggestions and prepare the media accordingly.

Make sure that the culture media are clear. There must be no sediment and no flocculency. Not infrequently the medium fails to become sufficiently clear, even though every precaution has been taken. In such cases clarification may be tried, rather than to discard it. Add the white of an egg, thoroughly beaten, to a liter of the medium in the liquid state and at a temperature below the coagulating point for albumen, mix thoroughly; boil for ten minutes, and filter. The coagulating albumen takes up the impurities which remain upon the filter with the albumen, while the medium comes through perfectly clear. Media which have become infected with bacteria as the result of inadequate setrilization should be discarded. Do not attempt to clarify them. They may become clear, but they are nevertheless objectionable because of the substances which the bacteria may have liberated and which might interfere with the development of the bacteria to be grown in it subsequently.

FIG. 25.—Koch safety burner. Should the flame be blown out, an automatic devise shuts off the gas.

Most of the tubes with solid media (Loeffler's serum, gelatin, agar, and gelatine-agar) should be slants. The slanting surface offers certain advantages in making diagnostic bacterial cultures. The usual, non-slanting tubes, for deep stab cultures, should, however, also be held in readiness. Keep all tubes in suitable containers, in a dry, cool, clean place. To guard against infection by mould and other organisms, it is well to cap all tubes with the rubber caps or the tin foil dipped in corrosive sublimate and paraffin, as already suggested. In case of liquid media, the rubber stoppers or the rubber caps are much preferred, or the hot paraffin may be painted over the tin foil and upper end of tube by means of a small brush. Apply two or three coats. Thus protected, there is no danger of outside infection.

The chances are that the physician who calls for tube culture media will also require the use of an incubator. This the pharmacist should have in readiness. The usual copper double-walled water-jacket incubator, with thermo-regulator, kept at a temperature of about 25° C., will serve the purpose.

The swab to be supplied with each tube of slanted Loeffler's serum consists of a piece of wire or of pine wood four inches long, around the

lower end of which a pledget of absorbent cotton has been wound and firmly tied by means of thread. This is placed in a test-tube, which is then plugged with cotton and sterilized in the dry sterilizer (one hour at a temperature of 140° C.) The physician wipes the cotton end of the swab over the suspected throat area, and then lightly rubs it over the surface of the serum

FIG. 26.—Hot water funnel with stand and ring gas burner.

FIG. 27.—Hot water funnel with stand.

tube slant. The swab is returned to the tube, the cotton plug is restored and then returned to the board of health to be destroyed in stove or furnace fire, or destroyed by the attending physician in case there is no board of health to recieve it.

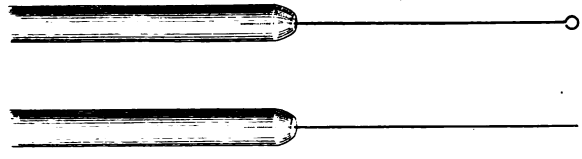

FIG. 28.—Glass rods with platinum wire, straight and loop, for inoculating culture tubes, Petri plates, etc.—(*Williams.*)

## 7. Making Bacterial Cultures.

This branch of the science of bacteriology is of comparatively little importance to the pharmacist. While it is desirable to know what bacterial cultures are and how to make some of them, it is wholly unlikely that the pharmacist will be called upon to do extensive work along this line. This is

the work of those who make bacteriology a specialty. Such bacterial cultures as are likely to come to the notice of pharmacists will most generally be prepared by physicians, health officers, and other specialists in bacteriology. The pharmaceutical bacteriologist *may* be called upon to make bacterial examinations of drinking water, of milk, of ice cream, and other food materials; of syrups, liquors, aquæ, tinctures, fluidextracts, infusions, etc., and he should, if possessed of some skill and adequate laboratory facilities, be able to do so.

The prime object in growing bacteria in artificial culture media is to make possible their further more careful and more extended study. The

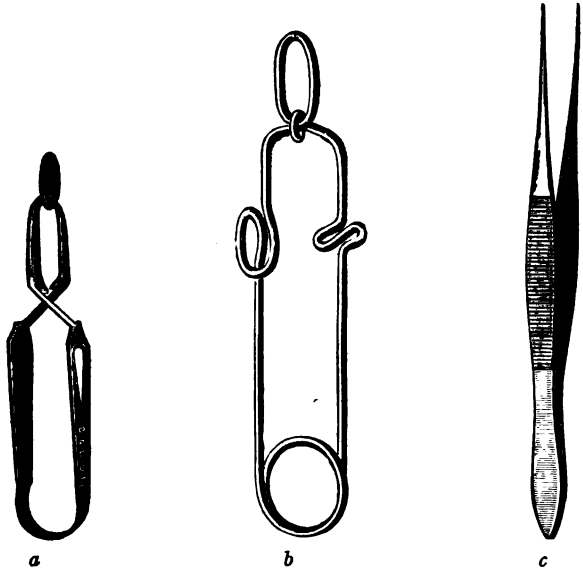


FIG. 29.—Cover-glass pincers. *a* and *b* are self-clamping but the pressure is often enough to break thin covers.

study of bacteria in their natural or normal surroundings is all-important, but is not complete without the artificial culturing.

As a rule, bacteria are biologically associated with other organisms, and it is unusual to find pure cultures in nature or in natural media. An open sore may contain several or many species and varieties of bacteria, in addition to the pus germs. The intestinal tract of the typhoid patient contains bacteria other than the comma bacillus of Koch. The tubercular bronchials always show a mixed infection. The diphtheric membrane contains some foreign germs, etc. Some infections, particularly those of internal tissues or organs, as lymphatic glands for example, may present practically pure cultures. However, no matter how mixed an infection

may be, there is always a predominating type present, or, to state it more correctly, it is the unusual development of the predominating type which determines the diagnostic characteristics of the infection.

It must also be borne in mind that bacteria behave differently when taken out of their natural environment and placed in artificial culture media. It does not at all follow that, in the case of a mixed infection, the predominating and diagnostic microbe will remain the predominating type when said mixed infection is transferred to some artificial culture medium. In fact, the predominating microbe may develop very slowly or with great difficulty, if at all, in the artificial culture media; whereas one or more of the associated microbes may thrive remarkably well, soon entirely overshadowing the former. These and other conditions occasion some of the great difficulties encountered in determining the primary causes of some microbic and protozoic diseases and infections.

FIG. 30.

A. *Test-tube Cultures.*—Inoculate several test-tubes, containing nutrient gelatin or agar gelatin, with any

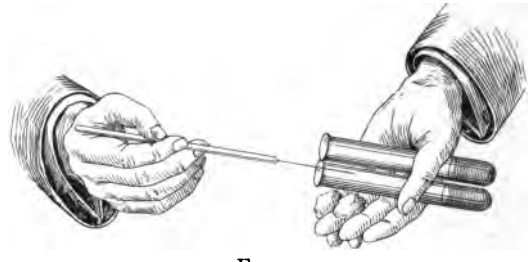

FIG. 31.

FIG. 30.—Cotton plugged tube with a potato slant resting on a bit of glass rod to keep the potato out of the water in the bottom of the tube. (*Williams.*)

FIG. 31.—Manner of holding tubes when making subcultures. The cotton plugs, removed from the two tubes, should be held in hand holding the platinum rod, as explained in the text. (*Williams.*)

material which is known to be bacterially infected. This is done by touching the infected material with the tip of a heat-sterilized (by holding in flame of Bunsen burner until red hot) platinum needle (prepared by fusing a platinum wire, 1 1/2 inches long, into the end of a glass rod, six to seven inches long), then removing the cotton plug from the test-tube, and pushing the needle, carrying the microbes, into the culture medium down to the very bottom of the tube. Replace the cotton plug at once, pass the needle into the flame of the Bunsen burner until red hot, to sterilize it, and lay aside for the next tube inoculation. This is known as a deep stab tube inoculation. In this manner inoculate some five or six tubes.

Also make streak inoculation in tube slants by simply passing the infected platinum needle over the middle of the tube slant surface, from lower end toward the top, observing the instructions regarding the cotton plug and needle sterilization, with each tube inoculation. Number the tubes serially, and in a special notebook make entry of all desirable data pertaining to each inoculation, making such entries under each tube number. Place tubes vertically in a suitable holder, as tumbler, beaker, wire basket, etc., and set aside in incubator or in some container to which you alone have access.

In warm weather the first bacterial growths may appear at the end of thirty-six hours. In cold or cool weather nothing may appear for two, three, and even four to five days. Note the nature of the bacterial growth in a deep stab inoculation and in the streak inoculation, as to

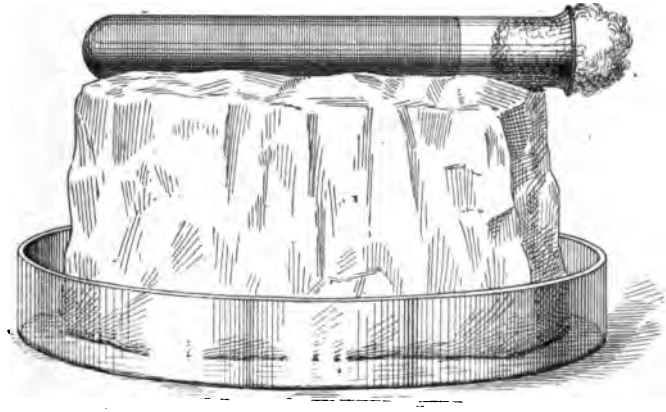

FIG. 32.—Making an Esmarch roll-tube culture. A lump of ice is placed in a dish and the inoculated tube is placed horizontally in a groove in the ice and revolved until the medium is well set. The groove may be made with test-tube full of hot water. (*Williams.*)

a. *Growth*—scanty, moderate, abundant, slow, rapid.

b. *Form of growth*—outline clearly defined, spreading, rugose, beaded, etc.

c. *As to surface*—flat, raised, concave, convex.

d. *Color*—translucent, glistening, waxy, transparent, opaque, light, chalky white, grayish-white, dark red, green, blue, yellow, lemon color, purple, etc.

e. *Odor*—comparative description.

f. *Consistency*—viscid, slimy, stringy, membranous, friable or brittle, dry, watery, etc.

g. *Changes in medium*—gelatin liquefied, gelatin not liquefied; colored, as grayed, browned, reddened, blued, etc. In case indicators are used, the possible color changes should be noted.

h. *Deep stab culture*—where is growth most active? If at bottom, it indicates anaërobic tendencies. If limited to top of medium, it indicates decidedly aërobic tendencies. (Most bacteria are decidedly aërobic; that is, they require oxygen to thrive.)

The test-tube cultures do not necessarily represent pure cultures, and the student cannot know whether the growths in the test-tubes represent the predominating bacterial flora in the substance from which the inoculations were made. The chief object in making the above cultures is to enable the student to get practice in this preliminary work, particularly as to making the cultural observations above indicated.

FIG. 33.—Kitasato filter for filtering hypodermic solutions, culture media, sera, water, etc. The material to be filtered is placed in the globose container and forced through the clay (infusorial earth) tube (Berkefeld filter bougie) by connecting the receiver with a vacuum pump. All parts of the filter must, of course, be sterilized by heat before and after using. (*Williams.*)

The student should now make transfers (subcultures) from the first tube cultures into second tubes, and note whether or not the characteristics originally noted are continued or repeated. If the transfer cultures are the same as the originals, it is an indication that the first cultures were pure (representing one species or variety), which is generally the case, though it must be borne in mind that one and the same species of microbe may undergo considerable change in extended culturing, as indicated in the changed culture characters. In fact, some of the changes are so extreme as to confuse even the most expert bacteriologists.

B. *Isolating Bacteria by the Plate Method.*—In order to seperate or isolate the several species and varieties of bacteria in any contaminated substance, it is only necessary to dilute the inoculating material sufficiently. For this purpose there is necessary, sterilized Petri dishes containing heat-sterilized gelatin or other solid media through which the bacteria from the contaminated substance are disseminated in numbers so small that the colonies from each and every microbe present may be visible to the naked eye (or aided by a simple lens). This is done as follows:

FIG. 34.—Streak culture on agar in a Petri dish. (*Delafield* and *Prudden.*)

To obtain isolation cultures of air bacteria it is only necessary to expose the Petri dish (with a layer of gelatin or agar-gelatin medium, sterilized) for about two minutes, immediately closing the dish and setting it aside to await developments. Making isolation cultures from contaminated solids or liquids is not quite so simple. Proceed as follows: Liquefy the gelatin in four or five test-tubes and keep them at a temperature of not more than 30° C.,

just high enough to keep the contents liquid; set them in a beaker filled with warm water (30° C.) until needed. Number the tubes from 1 to 5.

Dip a platinum loop (bend the end of a straight needle into a small loop) into the infected liquid, as bouillon, milk, water, tea, syrup, tincture, fluid-extract, etc., etc., and pass one loopful into tube No. 1 (sterilize loop and return to its proper place). Rotate tube (replugged with the cotton and held vertically) rapidly between the hands for twenty seconds, to mix contents. By means of the platinum loop take two loopfuls (one loopful may serve) from tube No. 1 (which you have just inoculated and rotated) and pass them into tube No. 2. Plug both tubes, set aside tube No. 1, and rapidly rotate tube No. 2. Take two loopfuls from tube No. 2 and transfer to tube No. 3, and proceed as before. Now pour contents of tube No. 1 into a sterile

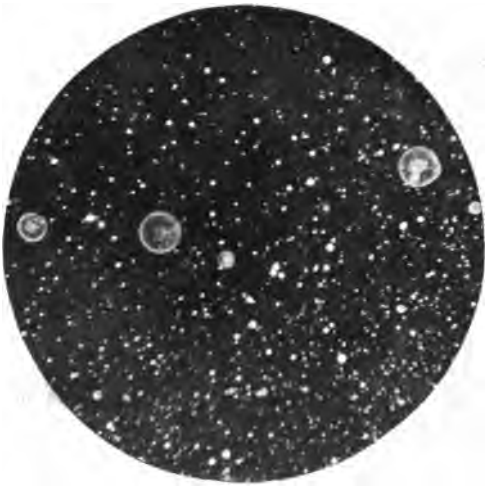

FIG. 35.—Appearance of colonies on gelatin in a Petri dish. Differences in size of colonies may indicate different species. Differences in color also indicating different species, cannot be shown in the figure. (*Williams.*)

Petri dish, also numbered 1; contents of tube 2 into Petri dish 2; and tube 3 into Petri dish 3. Wait until the media in the Petri dishes are solidified, and then set aside at the room temperature to await developments. In the course of two or three days it will perhaps be found that very many minute specks are visible in dish No. 1, some one hundred or more may appear in dish No. 2, and perhaps not more than ten or twenty in dish No. 3. Observe carefully the several growths in dishes 2 and 3. Each visible growth indicates the development from a single microbe. Are the several growths all alike, or do they differ? Differences in color and in outline of growths indicate different species of bacteria. The several different kinds of bacteria

may now be transferred to test-tubes by means of the straight platinum needle or the loop, and the observations may thus be extended. Transfers can be made to different kinds of media, as agar, gelatin, agar-gelatin, beef broth, milk, prepared potato, etc.

C. *Making Bacterial Counts.*—In order to determine the number of bacteria in any given substance the same procedure as was just described is followed, with the difference that a definite amount of the thoroughly mixed contaminated substance is added to a definite amount of culture medium in the test-tubes in which the dilution mixtures are made. For example, we will suppose that it is desired to determine the number of bacteria (per c.c.) in milk: Thoroughly mix the sample of milk by shaking it in the container. Take 0.1, 0.2, 0.5, or 1 c.c. of the milk (by means of a sterilized graduated pipette) and add it to 9 c.c. of the liquefied culture medium in tube No. 1; 1 c.c. of tube No. 1 to tube No, 2, also with 9 c.c. of medium; 1 c.c. of tube No. 2 to tube No. 3 (with 9 c.c. of medium), following the other directions as already given. Plate out as already explained, and watch developments. In Petri dish No. 1 the number of bacterial growths (colonies) will no doubt

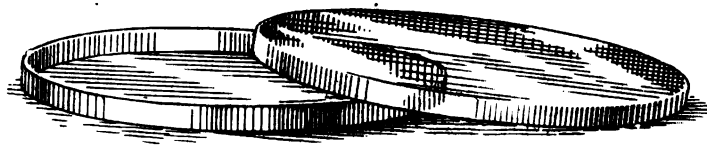

FIG. 36.—Petri dish. These dishes are among the essentials in the bacteriological laboratory. (*Williams.*)

be so great as to make counting impossible. Petri dish No. 2 may contain 360 colonies, and dish No. 3 may contain not more than 40. An average is obtained by repeating the test (using the same milk sample) a number of times. In the above milk sample the average may be 42,000 microbes per c.c. If the bacterial content is high, it is necessary to extend the attenuation four and even five times.

If it is desired to determine the number of bacteria per gram of dry soil, it will be necessary to carefully weigh a small quantity (1 gm., more or less) of average soil, triturate the entire sample with say, 100 c.c. of sterile distilled water, and from this make the dilution cultures as above described, using 1 c.c. or less of the soil triturate. To compute the number of bacteria per gram of *dry* soil, it will now be necessary to determine the moisture percentage in a sample of soil taken from the same place as the sample which was used in making the triturate. The solution is simple. We will suppose the triturate sample weighed 0.856 gm. and the number of bacteria found was

3,000,000; and the percentage of moisture was 10. From these data it would be found that 1 gm. of dry soil will contain 3,855,011 microbes.

The above is sufficient to make clear how one might proceed to determine the number of microbes in and upon old pills, tablets, powders; on one ivory vaccine tip, in one glycerinated vaccine tube, in 1 c.c. of bacterial vaccine, antitoxin, syrup, tincture, fluidextract, camphor water, distilled water, sewage, drinking water, etc. Naturally, great caution and care must be observed to avoid errors and faulty conclusions. In fact, no one should attempt such work in actual practice until after considerable preliminary laboratory experience.

It is not practicable nor is it necessary to give fuller information regarding bacterial cultures. We have not touched upon the various methods for determining whether or not the microbes under investigation are essentially

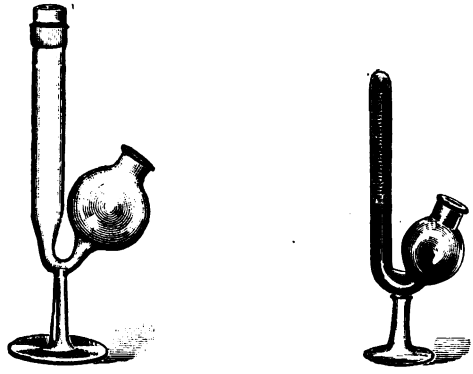

FIG. 37.—Graduated fermentation tube. These tubes are required for gas determination with colon bacillus and other gas-forming micro-organisms.

aërobic or essentially anaërobic; the manner of determining the thermal death-point; relationship of rate of growth to temperature, etc. We have said nothing of the use of indicators added to culture media, as litmus, rosolic acid, and phenolphthalein, nor have we explained the special use of special culture media in determining the nature and identity of bacteria. These and many other details we must omit, merely stating that, should it become desirable to make such investigations, the necessary information must be secured elsewhere, as in some standard laboratory guide in bacteriological technic.

The following outline of special methods will serve as a guide in making bacteriological examinations of soils, air, pharmaceuticals, liquids, etc.

D. *Culturing Soil Bacteria.*—Soil is a mixture of dead and decayed organic matter, sand and living organisms and their spores. Near the surface the soil contains large numbers of bacteria, from 10,000 to 10,000,000 per gram,

and more. In fact the fertility of the soil is practically proportional to the number of bacteria present. Most species of soil bacteria are harmless to man though the bacilli of tetanus (lockjaw), of typhoid fever, of malignant edema, of anthrax, and of pus formation may be present. The tetanus germ is quite common in garden soils and the anthrax germ is apt to occur in cattle pens, pastures and other places frequented by cattle. Other soil bacteria are decidedly useful as will be more fully explained elsewhere.

Some soil bacteria (the nitrifiers) do not grow on the usual media while others thrive exceedingly well in such media. Anaërobic forms must be cultured in the absence of air or oxygen.

The root nodule bacteria of the leguminosæ can be grown readily on gelatin or agar. The tubercles or nodules must be thoroughly cleansed and repeatedly washed in boiled distilled water, then rinsed for ten seconds in a 1–1000 corrosive sublimate solution, and finally thoroughly rinsed (three minutes) in boiled distilled water. Crush several of the sterilized nodules in a sterile watch crystal, by means of a sterile glass rod and from this make the dilution plate cultures and set aside at room temperature. Colonies of small motile bacteria (*Rhizobium mutabile*) will appear in about four days.

To test the soil bacterially, select thoroughly mixed samples and plate out as already suggested, using every precaution to prevent the introduction of extraneous germs. Cultures can also be made from internal plant tissues by following, in general, the directions given under root nodule bacteria, excepting that after the washing and rinsing, the root, instead of being crushed, is cut or broken across and the inoculation material is taken from the inner tissue by means of a platinum needle or scalpel.

E. *Bacteria of the Air.*—Air currents carry the germ-laden dust and dirt particles. The number and kind of air bacteria depends upon environment, climatic conditions, moisture, sunlight, etc. The air currents are the main factors in germ dissemination. Spores and dry (though not dead) bacilli may be carried many miles. Air microbes are derived from the soil surface and from the objects surrounded by the air. Bacteria are exhaled with the breath and are carried and distributed from and by animals, plants and clothing. The air carries pus germs, tubercle bacilli, anthrax bacilli and their spores, besides other pathogenic microörganisms, including also yeast cells and the spores of higher fungi.

Air microbes may be studied by exposing a Petri dish containing sterilized agar or gelatin, for two minutes or longer. The number of colonies that will appear will depend upon the locality, season, air moisture, etc. To determine the number of microbes in a given volume of air the Sedgwick-Tucker aërobioscope is used, though similarly constructed apparatus may be made by any fairly skillful student. The aërobioscope consists of a glass cylinder as shown in the illustration. The open ends are plugged with cotton.

Granulated sugar is loosely packed into the narrow end and all is then sterilized in a hot-air sterilizer (not over 120° C.). Pass a given quantity of air through the aërobioscope by attaching an aspirator bottle to the narrow end and allowing a given volume of water to run out of the bottle. The volume of air drawn through equals the volume of water run from the bottle. Of course the cotton plug is removed from the larger end of tube while the water is running. The bacilli and spores are caught in the sugar, while the air passes through. Replace cotton plug and shake the sugar into the larger end of tube. Remove cotton plug again and pour in about 10 to 15 c.c. of liquefied (40° C., not hot) gelatin. Roll the tube held horizontally. The gelatin dissolves the sugar and mixes with it. Roll on ice to hasten the hardening of the gelatin. Set aside in incubator, at room temperature (20° C., about). The number of colonies which appear indicates, approx-

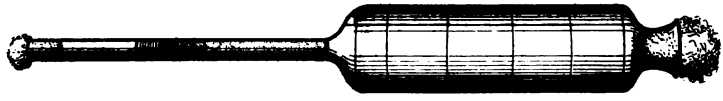

FIG. 38.—Aerobioscope after Sedgwick-Tucker, plugged with cotton. The larger end in which the cultureing is done is ruled to facilitate the counting of colonies.

imately the number of microbes in the volume of air aspirated. Let us suppose that the number of colonies was 125, the volume of air aspirated 10 liters, from which we would get 1250 bacteria per cubic meter of air.

F. *Bacteria of Liquid Substances.*—The bacteria of water, milk, tinctures, fluidextracts, aquæ, aërated waters, mineral waters, distilled water, broth, and liquids generally, can be studied quantitatively in a comparatively simple manner. By means of a sterile 1 c.c. graduated pipette, run 0.1 c.c. to 0.5 c.c. of the liquid into the center of a sterilized petri dish, pour upon this enough (about 10 c.c.) melted (sterile) agar or gelatin and mix by tilting the dish slightly from side to side. Set aside for the medium to harden and incubate at the room temperature, or at 25° C., if quicker results are desired. This method is satisfactory if the number of bacilli present is comparatively small. If very abundant, dilutions must be made in the manner already described.

The following general suggestions should be observed in making bacteriological determinations of liquids:

a. Containers for samples (other than the original containers) must be sterile and closed with sterile corks or cotton plugs. If the samples are to be carried any distance they should be packed in ice. In no case is it wise to keep a sample longer than forty-eight hours before culturing it. If the sample is to be examined within two or three hours after collecting it, placing on ice is not absolutely necessary.

b. Every sample should be thoroughly mixed before making cultures. Shake well, about twenty times. This is very important.

c. All glassware, pipettes, etc., must be thoroughly sterilized by washing, use of disinfectants, rinsing, wiping, hot air and steam sterilization, etc.

d. Incubate at room temperature, as a rule. Colonies will begin to appear in forty-eight hours. The maximum development will be in three or four days, in most instances, provided the temperature does not fall below 20° C.

e. As a rule the presence of abundant gelatin-liquefying organisms may be looked upon with suspicion. Certain sewage organisms liquefy gelatin very actively.

f. The colon bacillus and some sewage cocci give pink colonies with lactose litmus agar medium. The cocci colonies are a deeper vermilion than the colon colonies. Sewage-contaminated water will show many pink colonies.

g. Certified milk (just delivered) should not show more than from 1000 to 10,000 colonies per c.c.

h. Wholesome uncertified milk should not show more than from 30,000 to 50,000 colonies per c.c. The number of colonies permissible varies in different states and in different localities in the same state. The number of colonies may range from 25,000 to 1,000,000 per c.c., and even more, and yet the milk may be pronounced wholesome. No pink colonies should be present. No pus cells should be present (centrifugalized sediment).

i. Good drinking water should not show more than 50 to 100 colonies per c.c. and there should be no pink colonies, only a few liquefying colonies (1–10) and most of the colonies should develop best at 20° C. If 50 per cent. of the colonies develop best at 30° to 38° C. this indicates probable sewage contamination or contamination with intestinal bacteria. This differential temperature test is considered of importance in the bacterial examination of drinking waters. Normal water gives a proportion of 1 colony of high temperature organisms to from 25 to 50 colonies of low temperature organisms. In sewage contaminated water the proportion is 1 to 4 and even less.

j. Thus far there are no standards for the bacteriological testing of pharmaceuticals. Tinctures and fluidextracts should show only few colonies per c.c., not over 30 to 60. Sera should show none. Well prepared and properly ripened small-pox vaccine should show only a few colonies per point or per glycerinated tube. Aquæ often show abundant colonies, from 10,000 to 10,000,000 per c.c. and more.

k. The colon bacillus should not be present in drinking water, in milk or in pharmaceuticals. If present, it indicates sewage or other objectionable contamination. The colon bacillus is motile in young broth cultures, forms

no spores, is gas- (dextrose broth cultures in fermentation tube) and indol-forming, reduces nitrates to nitrites, does not liquefy gelatin and is not stained by Gram's method.

l. Syrups of all kinds, unless very carefully prepared and carefully kept to prevent fermentation, are apt to show numerous bacteria, yeasts and moulds. Any syrup showing signs of yeast fermentation (gas bubbles, vinous odor) or mouldiness, is not fit for use and should be rejected. The attempt to render it usable by boiling is unsatisfactory, furthermore the changes produced by the organisms are always objectionable and cannot be rectified by heating or by other methods of sterilization.

m. Recent investigations have shown that many of the marketed (bottled) mineral waters contain numerous bacteria, from 10,000 to 300,000,000 and more per c.c. In some cases colon bacilli have been found. These findings prove that in many instances the methods of bottling must be careless or otherwise unsatisfactory since sewage contamination is not reasonably possible under proper sanitary conditions. Undoubtedly the contamination is in some instances due to reused and inadequately cleaned and sterilized containers and in other instances to impure and inadequately sterilized mineral water. A popular opinion prevails that the chemicals in the mineral waters are sufficiently germicidal to destroy bacteria but this is not the case. Bacteria may develop actively in a great variety of solutions of high concentration provided such solutions are chemically balanced. Loeb, Osterhaut and others have shown, for example, that ocean water is chemically balanced, thus being suitable to maintain life in a great variety of organisms.

G. *Bacteria in Canned Fruits.*—The work recently demanded by the pure food laws (federal and state) has shown that such food substances as canned fruits of all kinds, including jams, jellies, preserves, catsups, tomato pastes, etc., are frequently highly contaminated with yeast cells, moulds and their spores, and other higher fungi, and bacteria. It is, however, evident that the food products named may be quite free from such contamination as may be seen from the examination of canned food products prepared by the careful housewife. That manufacturers may approximate the home condition is demonstrated by the fact that factory products are found on the market, which are quite free from contamination.

Since wholesome ripe fruit contains yeast cells, bacteria and mould in very small numbers only, and since most of these organisms are removed in the various steps of the processing, as washing, peeling, steaming, etc., it is evident that the finished factory product should, like the home-made product, contain these organisms in negligibly small numbers only, provided, of course, that wholesome fruit is used. However, most of the factory samples thus far examined have shown numerous dead yeast cells, mould spores, mould hyphæ, and bacteria, indicating the use of fruit, fruit pulp, fruit

juices, fruit refuse, etc., which was decomposed or undergoing fermentation or decomposition prior to or at the time of manufacture. The organisms named prevail in varying amounts in different products. Yeast organisms are apt to predominate in jellies, fruit juices and fruit pulp; bacteria in catsups and pastes; and moulds in certain fruits as strawberries, blackberries and raspberries.

The presence of numerous dead yeast cells (1,000,000 to 50,000,000 per c.c.) is evidence that the material was undergoing alcoholic fermentation just prior to or at the time of manufacture. Tomato pastes have been found on the market showing over 400,000,000 bacteria per c.c. besides numerous yeast cells and considerable mould. The bacterial content of catsups is apt to run high, from 10,000,000, to 50,000,000 and more per c.c. Not

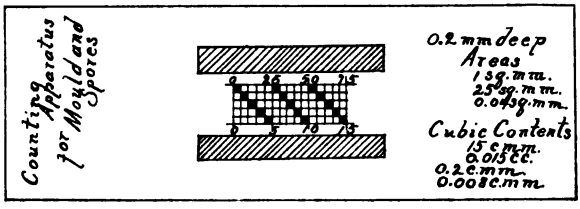


FIG. 39.—Counting apparatus for mould, yeast cells and spores. From the measuring values marked on the slide it is easy to determine the number of mould hyphal clusters, yeast cells and spores per c.c. of the substance under examination. Used with No. 2 ocular and No. 3 and No. 5 objectives.

The rulings are as follows: There are 75 square millimeters in the entire area, of three squares of 25 square millimeters each. The one-millimeter areas are to be used in determining the quantity of mould, dirt, sand and other impurities present. The one-millimeter areas indicated black in the figure are marked off into 1/25 (0.04) square millimeters. These smallest areas are used in making spore and yeast cell counts. The depth is 0.2 millimeter.

We will suppose that a given fruit sample, as strawberry jam, shows 30 yeast cells in the smallest area (0.2 mm. $\times$0.04 mm. = 0.008 cm.), then 1 c.c. of the substance would contain 3,750,000 yeast cells.

including the vinegar bacteria, which are introduced into catsups and pastes, such high bacterial content is generally due to bacterial development during or after manufacture. The presence of mould organisms and their spores (other than penicillium) indicates the use of mould-infested fruit. Penicillium, which is entirely saprophytic in habit, may develop after manufacture, particularly on the surface of inadequately sterilized fruit products in containers not entirely filled.

"Swelling" of cans containing fruit products is generally due to yeast development though it may also be due to bacterial activity, and indicates inadequate sterilization of either the container or of the fruit or both. Examination will show the presence of living yeast cells, or bacteria, perhaps air bubbles, and the characteristic vinous odor of yeast may be noted.

Based upon such conditions as can be made to prevail in carefully operated factories, the following may be given as the limits of the number of organisms permissible in the fruit products under discussion.

a. Yeast cells, either living or dead, not to exceed 1,000,000 per c.c.

b. Mould spores not to exceed 50,000 per c.c.

c. Hyphal clusters and hyphal fragments not to exceed 10,000 per c.c.; or not over 50 per cent. of separate and distinct fields of view under the compound microscope should show hyphal clusters or hyphal fragments.

d. Bacteria (either living or dead but not including vinegar bacteria in products to which vinegar is added) not to exceed 5,000,000 per c.c.

The above figures apply only to fruit products supposedly made from comparatively fresh fruits and fresh fruit juices. The yeast, bacterial and spore counts are made with a Thoma-Zeiss hemacytometer (Turck ruling) using a No. 5 (1/5 in.) objective with No. 2 (1 in.) ocular.

H. *Quantitative and Qualitative Bacteriological Testing.*—The following will serve as a general outline of bacteriological analyses which may be made in food and drug laboratories. The substances which require such bacteriological examination include catsups, tomato pastes, vinegars, water supplies, mineral waters, milk, ice creams, any and all substances which are suspected to be sewage contaminated, etc., etc.

The sequence of processes here given bear a progressive relationship. Whether process II is carried out will depend upon the findings under I and whether III shall be undertaken will depend upon the findings under II. The essential facts to be ascertained are whether or not there is possible sewage contamination as indicated by the presence of the colon bacillus, sewage streptococci and possibly the typhoid bacillus. The typhoid agglutinating tests are apt to prove unsatisfactory. In most instances this test will be unnecessary as the presence of the colon bacillus is evidence that the food, drug or drink is contaminated with sewage and is hence unfit for human use.

I. *Direct Count.*—For this purpose the Thoma-Zeiss hemacytometer with Turck ruling[1] is used (No. 2 ocular with 1/5 in. objective) which can be secured from any bacteriological supply house. The instructions for using it can be obtained from the dealer, though the measuring values indicated on the hemacytometer are sufficient to indicate the manner of making the counts. The rulings generally used for bacterial countings are 1/25 sq. mm. × 1/10 mm. deep, making an area of 1/250 cu. mm., or reduced to decimal fractions, 0.04 sq. mm. × 0.1 mm. deep = 0.004 cu. mm. We will suppose that the average of 20 counts shows 5 bacilli, then 1 cu. mm. would contain 1,250 bacilli or 1,250,000 in 1 c.c.

The direct count is, in many instances, very unsatisfactory for several

[1] To render the ruled lines visible rub a very soft pencil over the ruled area.

reasons. Particles other than micro-organisms may be mistaken for bacilli or cocci and, furthermore, it cannot be known for a certainty that the organisms are dead or alive. If they are present in great abundance (10,000,000 to 100,000,000 and more per c.c.), ordinary smear preparations may be stained, using methyl blue or fuchsin. Dead bacilli, that is those which have been dead for some time, do not take the stain well, due to the fact that the cell-plasm is disintegrated.

Tomato pastes, anchovy pastes, catsups, some mineral waters and similar preparations, may contain bacteria in such numbers that dilutions are desirable or necessary to make counting possible. A dilution of one in ten will, as a rule, be sufficient. Weigh or measure one part (1 gm or 1 c.c.) of the substance, add it to nine parts filtered distilled water and mix thoroughly by shaking.

If the direct count shows bacilli in great numbers or if for any reason sewage contamination is suspected, and also to determine the number of living bacilli and spores suspected, proceed as follows:

II. *Plate Culture Counts.*—Make one set of plate cultures, using lactose litmus agar,[1] and incubate at 20° C. Make a second set of plate cultures, also upon lactose litmus agar, and incubate at 38° C. The usual dilution methods are followed when necessary, using preferably 0.1 c.c. quantities for the plates. This temperature differential test is considered of great importance. Colon bacilli and other micro-organisms, whose natural habitat is the intestinal canal, will develop actively at the higher temperature (38° C.), whereas the usual air, soil and water bacteria develop best at the lower temperature (20° C.). If the high temperature colonies approximate the low temperature colonies, sewage contamination may be suspected. If in addition many of the high temperature lactose litmus agar colonies show pink or light vermilion, the sewage contamination is practically proven. The colon bacillus, as well as sewage streptococci, give pink colonies, the latter being the brighter, more vermilion in coloration, due to the formation of acid (in the fermenting lactose). Examine the pink colonies under the microscope. The colon microbe is rod-shaped, rather thick, non-sporing, and shows motility in recent broth cultures, whereas the streptococci are smaller and are not rod-shaped. High temperature colonies as compared with low temperature colonies should not exceed 1:100 or 1:25. If the proportion is 1:4 or less, sewage contamination is very likely. After 36 hours the pink colonies may turn blue, due to the development of ammonia and amines.

Naturally the high temperature colonies must be studied within twenty-four to thirty hours whereas the low temperature cultures require much more time, two to four days.

[1] Add 1 per cent. of lactose to the usual agar medium and enough tincture of litmus to give it a lilac tinge.

If the temperature and color differential tests indicate sewage contamination, then the following additional tests should be carried out.

III. *Indol Reaction and Gas Formula.*—The indol reaction has already been explained. The gas formula is determined as follows: To sets of four graduated fermentation tubes containing glucose bouillon and lactose bouillon, add 0.1, 0.2, 0.5, and 10 c.c. of the suspected liquid. If gas formation is observed the presence of colon bacilli may be suspected. If the 0.1 c.c. tubes show gas formation then the presence of colon bacilli may be assumed. Fill the bulb of a tube, showing gas formation, with a 2-per cent. solution of sodic hydrate, hold thumb tightly over the opening and mix contents by tilting back and forth carefully. The portion of gas absorbed is $CO_2$ whereas the unabsorbed portion is supposedly hydrogen. The colon bacillus shows a gas formation of 1/3 hydrogen. Of course the total volume of gas is recorded before the sodic hydrate is added.

FIG. 40.—Colon bacillus. This microbe is quite large, in the comparative sense, and is morphologically typical of the group bacillus. The flagellæ are few in number and comparatively long.

The gas formula with a positive indol reaction is practically conclusive as far as the presence of the colon bacillus is concerned. Add to this the other tests and we have presumptive evidence of sewage contamination, and any article of food or drink showing such contamination is unfit for human consumption.

The colon bacillus, the bacilli of the hog cholera group and others have the power of reducing neutral red; producing a greenish-yellow fluorescence. For this reaction use glucose bouillon to which has been added 1 per cent. of a 0.5 per cent. solution of neutral red. In examining milk, the pus cell and leucocyte count is considered important; centrifugalize 10 c.c. of milk for thirty minutes, pour off supernatant milk and mix residue with

0.5 c.c. normal salt solution and make counts of pus cells and leucocytes per c.c. from the amount (0.5 c.c.). Abundant pus cells and leucocytes indicate abscess or other pathological condition of milk ducts or glands. This test is, however, of little significance excepting in the hands of authorities on diseases of cows. It is stated that as many as 100,000 leucocytes per c.c. may occur in apparently healthy animals.

Gelatin-liquefying organisms may be looked upon with suspicion when found in milk, water and other liquid-food substances intended for human consumption, as has already been explained.

It should be borne in mind that the colon bacillus is one of a group of some fifteen or more species and varieties of closely related micro-organisms which resemble each other in the following particulars:

1. Do not form spores.
2. Do not liquefy gelatin.
3. Produce acid in milk and cause milk coagulation.
4. Produce acid and gas in glucose and lactose media.
5. Produce acid and gas in bile-salt-glucose broth.
6. Grow well in temperatures ranging from 38° to 42° C.

In differentiating the colon bacillus, remember that this organism is rod-shaped (2 to 3$\mu$ long by 0.5 to 0.6$\mu$ wide), is motile, produces indol, gives rise to pink colonies on lactose (or glucose) litmus agar and reduces neutral red glucose (or lactose) agar with a greenish-yellow fluorescence.

It should also be remembered that sewage is a highly complex substance and contains micro-organisms in great variety and in great abundance. Among the organisms present are species of *Spirillum*, *Vibrio*, *Proteus* and *Beggiatoa* in addition to the bacilli and streptococci already mentioned. The typhoid bacillus does not thrive well in sewage. The number of bacteria present in crude or ordinary sewage (domestic, city, hospital, mixed, etc.) ranges from 1,000,000 to 100,000,000 and more per c.c. The work of these organisms is to break down and render soluble and assimilable (for plants) the organic matter composing the sewage, thus assisting the work of rotting bacteria generally.

The following is a tabulation of the bacteriological testing that should be made of foods (including pastes, catsups, milk, ice creams, water supplies, mineral waters, alcoholic beverages, etc.) that may show an excess of bacterial growth or which may be sewage contaminated:

## BACTERIOLOGICAL EXAMINATION.

I. Direct Count.

1. Bacilli per c.c. . . . . .
2. Cocci, per c.c. . . . . .

II. Plate and Tube Cultures. (Lactose-litmus-agar.)
   1. Temperature differential test.
      a. (20° C.) Colonies per c.c. . . . . .
      b. (38° C.) Colonies per c.c. . . . . .
   2. Color differential test.
      a. Pink and yellow colonies per c.c. . . . . .
      c. Not pink or yellow colonies per c.c. . . . . .
   3. Colorless gelatin liquefying colonies per c.c.
   4. Neutral red reduction, + or −.
   5. Indol reaction, + or −.
   6. Gram stain behavior, + or −.
   7. Gas (hydrogen) formula.
III. Agglutinating tests for Typhoid Germs.

## 8. Staining Bacteria.

Staining consists of the infiltration of the cell-substance with solutions of various coloring materials obtained for the most part from the group of coal-tar derivatives known as the aniline dyes. As is generally known, different cells and different portions of one and the same cell react differently with the various dyes used. This peculiar behavior brings out contrasts in appearances which aid very materially in determining the morphological characters. The prime object, therefore, in using stains is to aid in the study of cell morphology. Different bacteria react differently with the several stains used. Some species take certain stains very readily, while they are quite indifferent to other stains. The vegetative cell stains much more readily than do the spores. In fact, spores are stained with great difficulty; however, after they are once thoroughly stained they hold the color persistently.

The dyes which may be used in bacteriologic work are of many kinds, differing as to color and as to staining powers with different cells, cell-contents, and cell-parts. They are usually classified as acid or basic. Eosin, acid fuchsin, and picric acid are acid stains, and are said to be diffuse in their effects, having no special affinity for any special cell structure. Fuchsin, methylene blue, and gentian violet are basic, and appear to have special attraction for bacteria and for plasmic and nuclear substances of cells generally, for which reasons they are most generally employed as bacterial stains. Fuchsin is, in fact, about the only efficient stain for endospores, while gentian violet and methylene blue are excellent stains for the bacterial cell-wall.

It is known that certain substances possess the property of preparing the bacterial cells in such a way as to induce them to take up the dye more

readily, thus intensifying the stain, as aniline oil and carbolic acid. Such substances are called mordants, and may be used separately or added directly to the stain itself.

Certain liquids or solutions remove the stain from the bacterial cell more or less readily, as water and alcohol, but more particularly solutions of acids. Such substances are quite generally employed for removing any excess of stain from the bacterial cell or from the matrix in which the bacteria are fixed or embedded. Acidulated (with HCl) alcohol is most commonly employed. Ordinarily, rinsing in a small stream of water is sufficient. Some bacteria resist the decolorizing process with acids more strongly than others, and are said to be acid fast or acid proof, as, for example, the bacilli of leprosy and of tuberculosis, while the great majority of species give up the stain very readily. It is a fact that one and the same species of microbe reacts variably with one and the same stain, depending upon a variety of causes. Moderate heat hastens and intensifies the staining.

For ordinary purposes a single stain only is used, but sometimes structural differences are more clearly shown by what is known as double or contrast staining. Take, for example, a spore-bearing microbe, as that of anthrax. The spores may be stained by means of carbol fuchsin; the entire cell, excepting the spore, can be completely decolorized in acidulated alcohol, and then methylene blue or gentian violet applied as the contrast stain. We then have a blue cell-wall with a red spore. However, the beginner is apt to be disappointed in his attempts at double staining; in fact, even the most skilled bacteriological technologists are apt to meet with small success, and generally rest satisfied with the use of the single stain.

The pharmacist will have comparatively little to do as far as the actual staining of bacteria is concerned. He should, however, be able to prepare the more important stains, mordants, and other solutions which may be required by the city or health board bacteriologist or the physician, and we shall therefore give the more commonly employed preparations.

A. *Stock Solutions.*—Make saturated solutions of the basic dyes (fuchsin, gentian violet, and methylene blue) in 95 per cent. alcohol. Keep these in glass-stoppered bottles in a cool, dark place, ready for use in preparing the stains. The stock solutions should in all intances be filtered before using. Secure the dyes from reliable dealers and in small quantities. Do not make up large quantities of stock solutions or stains proper, as they gradually deteriorate, particularly if exposed to light.

B. *Mordants.*—The principal substances used are aniline, carbolic acid, tannic acid, glacial acetic acid, ferrous sulphate, sodium hydroxide solution, chromic acid, and a few others. Those in general use are the two first named. The others have a more limited use in special cases.

1. *Aniline Water.*

| | |
|---|---|
| Aniline, | 2 c.c. |
| Distilled Water, | 98 c.c. |

Shake frequently, and finally filter several times through filter paper. It should be perfectly clear. This preparation deteriorates rapidly. Make up small amounts and keep in a dark place. It becomes worthless, even when observing all precautions, in a few weeks.

2. *Carbolic Acid-Solution.*

| | |
|---|---|
| Carbolic Acid, | 20 c.c. |
| Distilled Water, | 100 c.c. |

Filter. This mordant is rarely used by itself.

C. *Stains.*—We give here the more important stains, approximately in the order of preferred use.

1. *Loeffler's Methylene Blue.*

| | |
|---|---|
| Stock Solution (saturated) Methylene Blue, | 30 c.c. |
| 1 : 10,000 Sol. KHO in Dist. Water, | 100 c.c. |

Mix, shake, filter. This stain is much used as a general bacterial stain and in the examination of blood, pus, etc.

2. *Aniline Gentian-Violet.*

| | |
|---|---|
| Aniline Water, | 75 c.c. |
| Stock Solution Gentian-Violet, | 25 c.c. |

Mix, shake, filter. This is an excellent bacterial stain.

3. *Carbol-Fuchsin.*

| | |
|---|---|
| Stock Solution of Basic Fuchsin, | 10 c.c. |
| 5 per cent. Sol. Carbolic Acid, | 100 c.c. |

Mix, shake, filter. This is one of the most useful stains with the so-called acid-proof microbes. It is also a spore stain, and is the most commonly employed stain used in contrast or double staining. It is a comparatively slow stain, but is permanent.

4. *Gram's Stain.*

Gram's stain is used for diagnostic purposes, and is perhaps the best known stain in the entire field of bacteriological technic. Its value depends upon the fact that certain microbes, when stained and afterward treated

with a solution of iodine and washed in alcohol, give up the stain. Such microbes are known as Gram-negative, whereas those which do not give up the stain are said to be Gram-positive.

The method of using this stain is somewhat complicated, requires care, and, with a beginner, often yields disappointing results. Keeping in mind the following will minimize the disappointments:

a. Long-continued (one year or more) subcultures frequently lose the Gram-stain behavior.

b. Old cultures, that is, those which have been growing in the same medium for several days or more, as a rule do not stain characteristically. With such cultures the results are often neither negative nor positive, just enough to be confusing and perplexing.

c. The solutions used must be fresh. The gentian-aniline solution, as well as the iodine solution, deteriorates quite rapidly.

d. Do not overstain, and do not decolorize too long. Stop decolorizing as soon as no more violet color comes away.

In the Gram method two solutions are used, namely:

1. Aniline gentian-violet, and
2. Gram's iodine solution.

| | |
|---|---|
| Iodine, | 1 gm. |
| Potassium Iodide, | 2 gm. |
| Distilled Water, | 300 c.c. |

The method, briefly outlined, is as follows:

a. Spread the bacteria evenly and thinly over the cover-glass (the usual smear preparation). Stain with the aniline gentian-violet for from two to five minutes. Warming will hasten and intensify the staining. Wash in water to remove excess of stain.

b. Drop on the iodine solution and allow it to act for about one minute or until the preparation assumes a coffee-brown color. It may be desirable to apply the iodine a second time.

c. Wash off the excess of iodine in water and then decolorize by dropping on 95 per cent. alcohol. Tip the slide and allow alcohol to run over the preparation; continue until the violet color ceases to stream away.

d. Finally rinse in water and examine in water. If desired, dry and mount permanently in Canada balsam or some other suitable mounting medium.

e. A contrast stain, such as eosin, fuchsin, safranin, or Bismarck brown, may be used, following (c).

Keeping in mind the difficulties already referred to in using the Gram method, and the additional possible source of error due to the fact that one and the same microbe will stain but feebly at one time and very intensely

at another time, we now name the principal organisms which are Gram-positive or Gram-negative.

*Bacteria and other Organisms Stained by the Gram Method.*

Staphylococcus pyogenes aureus.
Staphylococcus pyogenes albus.
Streptococcus pyogenes.
Micrococcus tetragenus.
Micrococcus lanceolatus.
Bacillus diphtheriæ.
Bacillus tuberculosis.
Bacillus of anthrax.
Bacillus of tetanus.
Bacillus of leprosy.
Bacillus aërogenes capsulatus.
Oidium albicans.
Actinomyces (of actinomycosis).

*Bacteria not Stained by the Gram Method*

Diplococcus of meningitis (intracellular).
Diplococcus of gonorrhea.
Micrococcus melitensis.
Bacillus of chancroids (Ducrey's).
Bacillus of dysentery (Shiga's).
Bacillus of typhoid fever.
Bacillus of bubonic plague.
Bacillus of influenza.
Bacillus coli communis.
Bacillus pyocyaneus.
Bacillus of Friedlander.
Bacillus proteus.
Bacillus pyocyaneus.
Bacillus mallei (glanders).
Spirillum of Asiatic cholera.
Spirillum of relapsing fever.
Bacillus of pneumonia.

### 5. *Pappenheim's Stain.*

| | |
|---|---|
| Sat. Aqueous Sol. Methyl Green, | 50 c.c. |
| Sat. Aqueous Sol. Pyronine, | 15 c.c. |

Mix and filter. This is much used for staining bacteria in pus and other pathological secretions. The bacteria are stained a bright red, while the cell nuclei are blue to purple.

### 6. *Smith's Stain.*

| | |
|---|---|
| Stock Sol. Basic Fuchsin, | 10 c.c. |
| Methyl Alcohol, | |
| Formaldehyde, | each 10 c.c. |
| Distilled Water, | to make 100 c.c. |

Mix and filter. Let stand for twenty-four hours before using. Renew in three weeks. This stain is much used to distinguish between bacteria and nuclear substances. Allow the stain to act for from two to ten minutes.

### 7. *Flagella Staining.*

Care is necessary in staining flagellæ. Numerous methods have been recommended, but Pitfield's method, as modified by Muir, is perhaps the best and at the same time comparatively simple. The following solutions are required:

*a. Mordant.*

| | |
|---|---|
| Tannic Acid (10 per cent. Aq. Sol.), | 10 c.c. |
| Sat. Aq. Sol. Mercuric Chlor., | 5 c.c. |
| Sat. Aq. Sol. Alum, | 5 c.c. |
| Carbol-Fuchsin, | 5 c.c. |

Mix, shake, filter or centrifuge. This solution does not keep longer than one week.

*b. Stain.*

| | |
|---|---|
| Sat. Aq. Sol. Alum, | 10 c.c. |
| Stock Sol. Gentian-Violet, | 2 c.c. |

Mix, filter. Carbol-fuchsin may be used instead of gentian-violet. This stain will not keep longer than a few days.

The method is as follows:

1. Drop on mordant. Leave for one minute, with gentle heat.
2. Rinse in water for two minutes.
3. Dry carefully at slight warmth.
4. Stain for one minute with gentle heat.
5. Wash, dry, and mount in Canada balsam.

In making the cover-glass preparation, take a loopful from a young aqueous subculture of some motile bacillus and touch it on the carefully cleaned cover and allow the drop to spread by rotating and tilting the cover. Do not use the loop more than is necessary. Flagellæ are very delicate and easily destroyed. Dry very carefully, and do not pass through flame more than three times.

### 8. *Spore Staining.*

As already stated, spores (endospores) of microbes stain with great difficulty, for which reason a contrast is effected negatively; that is, the rest of the cell is quickly stained, leaving the unstained, highly refractive spore to appear like a bit of glass within the colored frame. This is in many ways the most satisfactory way of demonstrating the presence of spores. The spores may,

however, be stained by the usual acid-fast or acid-proof methods, care being observed in decolorizing. Stain with hot carbol-fuchsin for a few minutes, wash, and decolorize quickly with 3 per cent. hydrochloric acid in 95 per cent. alcohol, and then use a contrast stain, as gentian-violet or methylene blue. The red spores will then appear in the violet or blue frame.

### 9. *Capsule Staining.*

The gelatinous capsule of microbes is also stained with great difficulty, and requires special methods and experience to yield anything like satisfactory results. The methods of Welch and Hiss are quite satisfactory. The capsule is, however, generally visible without any staining because of the light contrast that naturally exists. Certain substances, as glacial acetic acid (Welch method), cause the capsule to enlarge and take up the stain more readily. Certain staining methods bring out the capsule of certain microbes, as, for example, the Gram method as applied to pneumonia sputum.

The Muir method is perhaps the best for capsule staining. It is as follows:

1. Stain in carbol-fuchsin for one-half minute, with gentle heat.
2. Wash lightly in alcohol (95 per cent.).
3. Wash well in water.
4. Flood with mordant of

| | |
|---|---|
| Sat. Aq. Sol. Mercuric Chlor., | 2 c.c. |
| Tannic Acid (20 per cent. Aq. Sol.), | 2 c.c. |
| Sat. Aq. Sol. Potassium Alum, | 5 c.c. |

5. Wash in water.
6. Wash in 95 per cent. alcohol, one minute.
7. Wash in water.
8. Stain with methylene blue for one-half minute.
9. Dehydrate in alcohol.
10. Clear in xylene, and mount in Canada balsam.

There are numerous other special stains and special staining methods, which need not be mentioned here. Should the pharmacist be called upon to prepare any of these, he will find full particulars in any standard work on medical bacteriology.

## 9. Studying Bacteria.

The complete study of any one species of microbe with a view to determining its identity is a long and tedious process. It involves a study of the organism in its natural element and in artificial culture media, and its behavior in animal inoculation tests, etc. Special apparatus, experimental animals (as rats, mice, guinea-pigs, dogs, etc.), and technical experience and

skill are necessary. Just what kind of observations are involved in such study is indicated in the complete method as outlined by the Society of American Bacteriologists Jan., 1908, which is hereby submitted for the benefit of those who may wish to acquaint themselves with such details. The glossary of terms should be carefully considered first of all. The decimal system for indicating group relationships of microbes (Table I) is most unique and is very convenient for active workers. Those interested will find the desired explanations of the methods and reagents mentioned, in any of the larger works on medical bacteriology and in bacteriological technology. It is not at all likely that the pharmacist will ever have occasion to make use of the special methods cited. He should nevertheless acquaint himself with them sufficiently to comprehend their application in the study of pathogenic bacteria.

Our bacteria nomenclature is in some confusion, and unless the methods of naming bacteria are corrected, the confusion is certain to become much greater. The trouble lies in the failure to define group or generic delimitations. The present generic terms, "bacillus" and "micrococcus," include too many species. We have a confusing and almost incomprehensible array of synonyms, of which those applied to *Rhizobium mutabile* may serve as an example. The different names that have been given to this organism may be arranged as follows:

Pasteuraceæ, Laurent.
Bacteria, Woronin, 1866.
Bakteroiden, Brunchorst and Frank, 1885.
Microsymbiont, Atkinson, 1893.
Spores or gemmules, Ward and Ericksson.
*Bacillus radicicola*, Beyerinck, 1888.
*Cladochytrium leguminosarum*, Vuellemin.
*Phytomyxa leguminosarum*, Schroeter.
*Schinzia leguminosarum*, Woronin.
*Rhizobium leguminosarum*, Frank, 1890.
*Rhizobium Frankii*, Schneider, 1892.
*Rhizobium mutabile*, Schneider, 1902.
*Pseudomonas radicicola*, Moore, 1905.

The above synonomy is also interesting because it indicates a most remarkable difference of opinion regarding the nature and identity of this root-nodule organism. Further, as the result of the wholly inadequate group delimitations we have such name-monstrosities as *Granulobacillus saccharobutyricus mobilis nonliquifaciens*, and *Micrococcus acidi paralactici liquifaciens Halensi*. Reform in nomenclature is very desirable, and it must come through a careful definition of generic groups based on physiological characters, rather than upon largely morphological characters, as is done now.

It is advised that the pharmacist refrain from experimenting with pathogenic organisms, excepting in so far as he may act in coöperation with the physician. When experimenting with pathogenic organisms the greatest caution is necessary to guard against autoinoculation and the spreading of disease. It should be made a rule to treat every microbe studied as though it were virulently pathogenic, capable of spreading an epidemic. Never expose a colony (plate culture, tube culture, etc.) in such a way as to permit the escape of the organisms into the air. Pour a disinfecting solution (5 per cent. carbolic acid) into cultures that are to be discontinued and then boil container and all, for thirty minutes, before washing and cleaning the glassware. Never forget to sterilize the platinum needle *before* and *after* making an inoculation or a culture transfer.

## DESCRIPTIVE CHART—SOCIETY OF AMERICAN BACTERIOLOGISTS. [1]

### Glossary of Terms.

*Agar Hanging Block*, a small block of nutrient agar cut from a poured plate, and placed on a cover-glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging rock.

*Ameboid*, assuming various shapes like an ameba.

*Amorphous*, without visible differentiation in structure.

*Arborescent*, a branched, tree-like growth.

*Beaded*, in stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

*Brief*, a few days, a week.

*Brittle*, growth dry, friable under the platinum needle.

*Bullate*, growth rising in convex prominences, like a blistered surface.

*Butyrous*, growth of a butter-like consistency.

*Chains*, short chains, composed of 2 to 8 elements. Long chains, composed of more than 8 elements.

*Ciliate*, having fine hair-like extensions, like cilia.

*Cloudy*, said of fluid cultures which do not contain pseudozooglea.

*Coagulation*, the separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

*Contoured*, an irregular, smoothly, undulating surface, like that of a relief map.

*Convex*, surface the segment of a circle, but flattened.

*Coprophyl*, dung bacteria.

*Coriaceous*, growth tough, leathery, not yielding to the platinum needle.

*Crateriform*, round, depressed, due to the liquefaction of the medium.

*Cretaceous*, growth opaque and white, chalky.

*Curled*, composed of parallel chains in wavy strands, as in anthrax colonies.

*Diastasic Action*, same as *Diastatic*, conversion of starch into water-soluble substances by diastase.

[1] Prepared by F. D. Chester, F. P. Gorham, Erwin F. Smith, Committee on Methods of Identification of Bacterial Species. Endorsed by the Society for general use at the annual meeting, January, 1908.

*Echinulate*, in agar stroke a growth along the line of inoculation, with toothed or pointed margins; in stab cultures growth beset with pointed outgrowths.

*Effuse*, growth thin, veily, unusually spreading.

*Entire*, smooth, having a margin destitute of teeth or notches.

*Erose*, border irregularly toothed.

*Filamentous*, growth composed of long, irregularly placed or interwoven filaments.

*Filiform*, in stroke or stab cultures a uniform growth along line of inoculation.

*Fimbriate*, border fringed with slender processes, larger than filaments.

*Floccose*, growth composed of short curved chains, variously oriented.

*Flocculent*, said of fluids which contain pseudozooglea, *i.e.*, small adherent masses of bacteria of various shapes and floating in the culture fluid.

*Fluorescent*, having one color by transmitted light and another by reflected light.

*Gram's Stain*, a method of differential bleaching after gentian-violet, methyl-violet, etc. The + mark is to be given only when the bacteria are deep blue or remain blue after counterstaining with Bismarck brown.

*Grumose*, clotted.

*Infundibuliform*, form of a funnel or inverted cone.

*Iridescent*, like mother-of-pearl. The effect of very thin films.

*Lacerate*, having the margin cut into irregular segments as if torn.

*Lobate*, border deeply undulate, producing lobes (see *Undulate*).

*Long*, many weeks or months.

*Maximum Temperature*, temperature above which growth does not take place.

*Medium*, several weeks.

*Membranous*, growth thin, coherent, like a membrane.

*Minimum Temperature*, temperature below which growth does not take place.

*Mycelioid*, colonies having the radiately filamentous appearance of mould colonies.

*Napiform*, liquefaction with the form of a turnip.

*Nitrogen Requirements*, the necessary nitrogenous food. This is determined by adding to *nitrogen-free* media the nitrogen compound to be tested.

*Opalescent*, resembling the color of an opal.

*Optimum Temperature*, temperature at which growth is most rapid.

*Pellicle*, in fluid bacterial growth either forming a continuous or an interrupted sheet over the fluid.

*Peptonized*, said of curds dissolved by trypsin.

*Persistent*, many weeks, or months.

*Pseudozoogleæ*, clumps of bacteria, not dissolving readily in water, arising from imperfect separation, or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zooglea.

*Pulvinate*, in the form of a cushion, decidedly convex.

*Punctiform*, very minute colonies, at the limit of natural vision.

*Rapid*, developing in twenty-four to forty-eight hours.

*Raised*, growth thick, with abrupt or terraced edges.

*Repand*, wrinkled.

*Rhizoid*, growth of an irregular branched or root-like character, as in *B. mycoides*.

*Ring*, same as *Rim*, growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

*Saccate*, liquefaction the shape of an elongated sac, tubular, cylindrical.

*Scum*, floating islands of bacteria, an interrupted pellicle or bacterial membrane.

*Slow*, requiring five or six days or more for development.

*Short*, applied to time, a few days, a week.

*Sporangia*, cells containing endospores.

*Spreading*, growth extending much beyond the line of inoculation, *i.e.*, several millimeters or more.

*Stratiform*, liquefying to the walls of the tube at the top and then proceeding downward horizontally.

*Thermal Death-point*, the degree of heat required to kill young fluid cultures of an organism exposed for ten minutes (in thin-walled test-tubes of a diameter not exceeding 20 mm.) in the thermal water-bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

*Transient*, a few days.

*Turbid*, cloudy with flocculent particles; cloudy flocculence.

*Umbonate*, having a button-like, raised center.

*Undulate*, border wavy with shallow sinuses.

*Verrucose*, growth wart-like, with wart-like prominences.

*Vermiform-contoured*, growth like a mass of worms, or intestinal coils.

*Villous*, growth beset with hair-like extensions.

*Viscid*, growth follows the needle when touched and withdrawn, sediment on shaking rises as a coherent swirl.

*Zoogleæ*, firm gelatinous masses of bacteria, one of the most typical examples of which is the *Streptococcus mesenteroides* of sugar vats (*Leuconostoc mesenterioides*), the bacterial chains being surrounded by an enormously thickened firm covering inside of which there may be one or many groups of the bacteria.

## NOTES.

(1) For decimal system of group numbers see Table 1. This will be found useful as a quick method of showing close relationships inside the genus, but is not a sufficient characterization of any organism.

(2) The morphological characters shall be determined and described from growths obtained upon at least one solid medium (nutrient agar) and in at least one liquid medium (nutrient broth). Growths at 37° C. shall be in general not older than twenty-four to forty-eight hours, and growths at 20° C. not older than forty-eight to seventy-two hours. To secure uniformity in cultures, in all cases preliminary cultivation shall be practised as described in the revised Report of the Committee on Standard Methods of the Laboratory Section of the American Public Health Association, 1905.

(3) The observation of cultural and bio-chemical features shall cover a period of at least fifteen days and frequently longer, and shall be made according to the revised Standard Methods above referred to. All media shall be made according to the same Standard Methods.

(4) Gelatin stab cultures shall be held for six weeks to determine liquefaction.

(5) Ammonia and indol tests shall be made at end of tenth day, nitrate tests at end of fifth day.

(6) Titrate with $\frac{N}{20}$ NaOH, using phenolphthalein as an indicator: make titrations at same time from blank. The difference gives the amount of acid produced.

The titrations should be done after boiling to drive off any $CO_2$ present in the culture

(7) Generic nomenclature shall begin with the year 1872 (Cohen's first important paper).

Species nomenclature shall begin with the year 1880 (Koch's discovery of the poured plate method for the separation of organisms).

(8) Chromogenesis shall be recorded in standard color terms.

## TABLE 1.

A NUMERICAL SYSTEM OF RECORDING THE SALIENT CHARACTERS OF AN ORGANISM

(GROUP NUMBER).

100.....Endospores produced.
200.....Endospores not produced.
10.....Aërobic (Strict).
20.....Facultative anaërobic.
30.....Anaërobic (Strict).
1.....Gelatin liquefied.
2.....Gelatin not liquefied.
0.1.....Acid and gas from dextrose.
0.2.....Acid without gas from dextrose.
0.3.....No acid from dextrose.
0.4.....No growth with dextrose.
.01.....Acid and gas from lactose.
.02.....Acid without gas from lactose.
.03.....No acid from lactose.
.04.....No growth with lactose.
.001.....Acid and gas from saccharose.
.002.....Acid without gas from saccharose.
.003.....No acid from saccharose.
.004.....No growths with saccharose.
.0001.....Nitrates reduced with evolution of gas.
.0002.....Nitrates not reduced.
.0003.....Nitrates reduced without gas formation.
.00001.....Fluorescent.
.00002.....Violet chromogens.
.00003.....Blue chromogens.
.00004.....Green chromogens.
.00005.....Yellow chromogens.
.00006.....Orange chromogens.
.00007.....Red chromogens.
.00008.....Brown chromogens.
.00009.....Pink chromogens.
.00000.....Non-chromogenic.
.000001.....Distasic action on potato starch, strong.
.000002.....Distasic action on potato starch, feeble.
.000003.....Distasic action on potato starch, absent.
.0000001.....Acid and gas from glycerin.
.0000002.....Acid without gas from glycerin.
.0000003.....No acid from glycerin.
.0000004.....No growth with glycerin.

The genus according to the system of Migula is given its proper symbol which precedes the number thus: (7)

BACILLUS COLI (Esch.) Mig. becomes .....B. 222.111102
BACILLUS ALCALIGENES Petr. becomes .....B. 212.333102
PSEUDOMONAS CAMPESTRIS (Pam.) Sm. becomes. .....Ps. 211.333151
BACTERIUM SUICIDA Mig. becomes .....Bact. 222.232103

## DETAILED FEATURES.

NOTE.—Underscore required terms. Observe notes and glossary of terms.

I. MORPHOLOGY (2)

1. *Vegetative Cells,* Medium used.....temp.....age.....days
   Form, *round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.*
   Limits of Size.....
   Size of Majority.....
   Ends, *rounded, truncate, concave.*

| | | |
|---|---|---|
| Hanging-block | Agar | Orientation (grouping)..... |
| | | Chains (No. of elements)..... |
| | | *Short chains, long chains.* |
| | | Orientation of chains, *parallel, irregular.* |

2. *Sporangia,* medium used.....temp.....age.....days
   Form, *elliptical, short rods, spindled, clavate, drum-sticks.*
   Limits of Size.....Size of Majority.....

| | | |
|---|---|---|
| Hanging-block | Agar | Orientation (grouping)..... |
| | | Chains (No. of elements)..... |
| | | Orientation of Chains, *parallel, irregular.* |

   Location of Endospores, *central, polar.*

3. *Endospores.*
   Form, *round, elliptical, elongated.*
   Limits of Size.....
   Size of Majority.....
   Wall, *thick, thin.*
   Sporangium wall, *adherent, not adherent.*
   Germination, *equatorial, oblique, polar, bipolar, by stretching.*
4. *Flagella,* No.....Attachment, *polar, bipolar, peritrichiate.* How Stained.....
5. *Capsules,* present on.....
6. *Zoogleæ,* Pseudozooglea.
7. *Involution Forms,* on.....in.....days at.....° C.
8. *Staining Reactions.*
   1:10 watery fuchsin, gentian-violet, carbol-fuchsin. Loeffler's alkaline methylene blue.
   Special Stains
   Gram.....Glycogen.....
   Fat.....Acid-fast.....
   Neisser.....

II. CULTURAL FEATURES (3)

1. *Agar Stroke.*
   Growth, *invisible, scanty, moderate, abundant.*
   Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
   Elevation of growth, *flat, effuse, raised, convex.*
   Luster, *glistening, dull, cretaceous.*
   Topography, *smooth, contoured, rugose, verrucose.*
   Optical Characters, *opaque, translucent, opalescent, iridescent.*
   Chromogenesis (8).....
   Odor, *absent, decided, resembling*.....
   Consistency, *slimy, buytrous, viscid, membranous, coriaceous, brittle.*
   Medium *grayed, browned, reddened, blued, greened.*

2. *Potato.*
   Growth, *scanty, moderate, abundant, transient, persistent.*
   Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
   Elevation of growth, *flat, effuse, raised, convex.*
   Luster, *glistening, dull, cretaceous.*
   Topography, *smooth, contoured, rugose, verrucose.*
   Chromogenesis (8).....Pigment in water *insoluble, soluble;* other solvents.....
   *Odor, absent, decided, resembling*.....
   Consistency, *slimy, butyrous, viscid, membranous, coriaceous, brittle.*
   Medium *grayed, browned, reddened, blued, greened.*
3. *Loeffler's Blood-serum.*
   Stroke *invisible, scanty, moderate, abundant.* Form of growth, *filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.*
   Elevation of growth, *flat, effuse, raised, convex.*
   Luster, *glistening, dull, cretaceous.*
   Topography, *smooth, contoured, rugose, verrucose.*
   Chromogenesis (8).....
   Medium *grayed, browned, reddened, blued, greened.*
   Liquefaction begins in.....d, complete in.....d.
4. *Agar Stab.*
   Growth, *uniform, best at top, best at bottom; surface growth scanty, abundant; restricted widespread.*
   Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent: liquefaction.*
5. *Gelatin Stab.*
   Growth, *uniform, best at top, best at bottom.*
   Line of puncture, *filiform, beaded, papillate, villous, plumose, arborescent.*
   Liquefaction, *crateriform, napiform, infundibuliform, saccate, stratiform;* begins in .....d, complete in .....d.
   Medium *fluorescent, browned*.....
6. *Nutrient Broth.*
   Surface growth, *ring, pellicle, occulent, membranous, none.*
   Clouding, *slight, moderate, strong; transient, persistent; none; fluid turbid.*
   Odor, *absent, decided, resembling*.....
   Sediment, *compact, occulent, granular, flaky, viscid on agitation, abundant, scant.*
7. *Milk.*
   Clearing without coagulation.
   Coagulation *prompt, delayed, absent.*
   Extrusion of whey begins in .....days.
   Coagulum *slowly peptonized, rapidly peptonized.*
   Peptonization begins on .....d, complete on .....d.
   Reaction, 1d....., 2d....., 4d....., 10d....., 20d.....
   Consistency, *slimy, viscid, unchanged.*
   Medium *browned, reddened, blued, greened.*
   Lab ferment, *present, absent.*
8. *Litmus Milk.*
   *Acid, alkaline, acid then alkaline, no change.*
   *Prompt reduction, no reduction, partial slow reduction.*
9. *Gelatin Colonies.*
   Growth *slow, rapid.*
   Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*

Elevation, *flat, effuse, raised, convex, pulvinate, crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate filamentous, floccose, curled.*
Liquefaction, *cup, saucer, spreading.*

10. *Agar Colonies.*
Growth *slow, rapid* (temperature .....).
Form, *punctiform, round, irregular, ameboid, mycelioid, filamentous, rhizoid.*
Surface *smooth, rough, concentrically ringed, radiate, striate.*
Elevation, *flat, effuse, raised, convex, pulvinate, umbonate.*
Edge, *entire, undulate, lobate, erose, lacerate, fimbriate, floccose, curled.*
Internal structure, *amorphous, finely-, coarsely-granular, grumose, filamentous, floccose, curled.*

11. *Starch Jelly.*
Growth, *scanty, copious.*
Diastasic action, *absent, feeble, profound.*
Medium stained.....

12. *Silicate Jelly (Fermi's Solution).*
Growth *copious, scanty, absent.*
Medium stained.....

13. *Cohn's Solution.*
Growth *copious, scanty, absent.*
Medium *fluorescent, non-fluorescent.*

14. *Uschinsky's Solution.*
Growth *copious, scanty, absent.*
Fluid *viscid, not viscid.*

15. *Sodium Chloride in Bouillon.*
Per cent. inhibiting growth.

16. *Growth in Bouillon over Chloroform, unrestrained, feeble, absent.*

17. *Nitrogen.* Obtained from *peptone, asparagin, glycocoll, urea, ammonia salts, nitrogen.*

18. *Best media for long-continued growth* .....

19. *Quick tests for differential purposes*.....
.....

## III. PHYSICAL AND BIOCHEMICAL FEATURES.

| 1. Fermentation tubes containing peptone-water or sugar-free bouillon and | Dextrose | Saccharose | Lactose | Maltose | Glycerin | Mannit |
|---|---|---|---|---|---|---|
| Gas production, in per cent. | | | | | | |
| $(\frac{H}{CO_2})$ | | | | | | |
| Growth in closed arm | | | | | | |
| Amount of acid produced 1d. | | | | | | |
| Amount of acid produced 2d. | | | | | | |
| Amount of acid produced 3d. | | | | | | |

2. *Ammonia production, feeble, moderate, strong, absent, masked by acids.*
3. *Nitrates in nitrate broth.*
   *Reduced, not reduced.*
   Presence of nitrites.....ammonia.....
   Presence of nitrates.....free nitrogen.....
4. *Indol production, feeble, moderate, strong.*
5. *Toleration of Acids: Great, medium, slight.*
   *Acids tested.*
6. *Toleration of NaOH: Great, medium, slight.*
7. *Optimum reaction for* growth in bouillon, stated in terms of Fuller's scale.....
8. *Vitality on culture media: Brief, moderate, long.*
9. *Temperature relations:*
   Thermal death-point (10 minutes' exposure in nutrient broth when this is adapted to growth of organism).....C.
   Optimum temperature for growth.....C.: or best growth at 15° C., 20° C., 25° C., 30° C., 37° C., 40° C., 50° C., 60° C.
   Maximum temperature for growth.....C
   Minimum temperature for growth.....C.
10. *Killed readily by drying:* resistant to drying.
11. *Per cent. killed by freezing* (salt and crushed ice or liquid air).....
12. *Sunlight:* Exposure on ice in thinly sown agar plates, one-half plate covered (times 15 minutes), *sensitive, not sensitive.*
    Per cent. killed.....
13. *Acids produced*.....
14. *Alkalies produced*.....
15. *Alcohols*.....
16. *Ferments: Pepsin, trypsin, diastase, invertase, pectase, cytase, tyrosinase, oxidase, peroxidase, lipase, catalase, glucase, galactase, lab, etc*.....
    .....
17. *Crystals formed*.....
18. *Effect of germicides:*

| Substance | Method used | Minutes | Temperature | Killing quantity | Amt. required to restrain growth |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |

## IV. PATHOGENICITY.

1. *Pathogenic to animals.*
   *Insects, crustaceans, fishes, reptiles, birds, mice, rats, guinea-pigs, rabbits, dogs, cats, sheep, goats, cattle, horses, monkeys, man.*
2. *Pathogenic to Plants:*
   .....
   .....
   .....
3. *Toxins, soluble, endotoxins.*
4. *Non-toxin forming.*
5. *Immunity bactericidal.*
6. *Immunity non-bactericidal.*
7. *Loss of virulence on culture-media: Prompt, gradual, not observed* in.....months.

## BRIEF CHARACTERIZATION.

Mark + or O, and when two terms occur on a line erase the one which does not apply unless both apply.

| | | | |
|---|---|---|---|
| (2) MORPHOLOGY | | Diameter over 1$\mu$ | |
| | | Chains, filaments | |
| | | Endospores | |
| | | Capsules | |
| | | Zooglea, Pseudozooglea | |
| | | Motile | |
| | | Involution forms | |
| | | Gram's Stain | |
| (3) CULTURAL FEATURES | Broth | Cloudy, turbid | |
| | | Ring | |
| | | Pellicle | |
| | | Sediment | |
| | Agar | Shining | |
| | | Dull | |
| | | Wrinkled | |
| | | Chromogenic | |
| | Gel. Plate | Round | |
| | | Proteus-like | |
| | | Rhizoid | |
| | | Filamentous | |
| | Gel. Stab. | Curled | |
| | | Surface-growth | |
| | | Needle-growth | |
| | Potato | Moderate, absent | |
| | | Abundant | |
| | | Discolored | |
| | | Starch destroyed | |
| | | Grows at 37° C | |
| | | Grows in Cohn's Sol | |
| | | Grows in Uschinsky's Sol | |
| BIOCHEMICAL FEATURES | Liquefaction | Gelatin (4) | |
| | | Blood-serum | |
| | | Casein | |
| | | Agar, mannan | |
| | Milk | Acid curd | |
| | | Rennet curd | |
| | | Casein peptonized | |
| | | Indol (5) | |
| | | Hydrogen sulphide | |
| | | Ammonia (5) | |
| | | Nitrates reduced (5) | |
| | | Fluorescent | |
| | | Luminous | |

| | | |
|---|---|---|
| DISTRIBUTION | Animal pathogen, epizoon | |
| | Plant pathogen, epiphyte | |
| | Soil | |
| | Milk | |
| | Fresh water | |
| | Salt water | |
| | Sewage | |
| | Iron bacterium | |
| | Sulphur bacterium | |

A. *Counting Plate Colonies.*—If the colonies in a Petri dish culture are few, not exceeding fifty to one hundred, they may readily be counted in full. If the colonies are quite numerous, the counting may be made easier by marking off (by means of a grease pencil or chalk) the bottom of the plate into two right angled cross-lines (quarter sectors) and these again into equal parts (1/8 sectors). Or one of the recommended special counting plates may be used. Either the square or circular plate will answer the purpose (see figures). When colonies are very numerous (200 and more) in a plate culture and quite uniformly distributed, it is not necessary to count them all. Count the colonies in a number of squares or sector areas (square centimeters) and multiply the average of twenty counts by the number of squares representing the entire surface area of the culture plate. As a rule the counting should be complete, however.

From the plate counts it is possible, by simple mathematics, to determine the number of microbes in the dilution cultures of water, milk, tinctures, fluidextracts, etc., as has already been explained.

*Studying Plate Colonies.*—The plate colonies should be studied macroscopically and also with the aid of a pocket lens and under the low power of the compound microscope. Place the dish on the stage of the microscope and focus upon the colonies carefully by means of the coarse adjustment. Note color, outline and other characteristics of the colonies, etc., as already set forth under tube cultures and in the official methods of the Society of Bacteriologists.

B. *Making Tube-cultures* (Subcultures).—Inoculate test-tubes (containing gelatin, agar or other media) with such colonies as it is desired to study further. This is done as follows: Hold the test-tube to be inoculated in left hand. Take up the platinum needle (straight or loop) in the right hand and pass the entire needle and glass rod (excepting the end held) through the flame of a Bunsen burner several times; heat the needle to a glowing red for a few seconds and then allow it to cool a few seconds. Lift the cover of the Petri dish high enough to pass the needle under, touch end of the platinum needle (straight or loop) on colony desired; let the dish cover

drop into place again; remove the cotton plug from test-tube by grasping it between two fingers (back of fingers toward the test-tube); make the inoculation (deep stab, shallow stab, or streak); withdraw needle; replace cotton plug; hold needle in flame until glowing red. To prevent the sputtering of the material on the end of the needle, hold near flame until dry and then heat

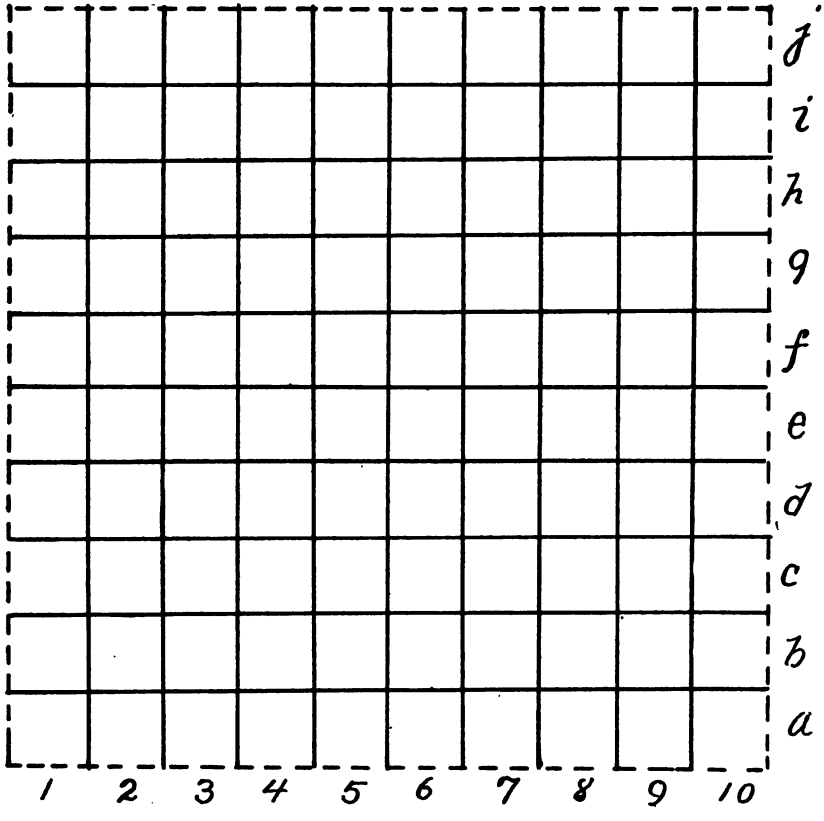


FIG. 41.—Square counting plate. Each area is one square centimeter. This is a simple as well as staisfactory counting plate to be used in counting Petri dish colonies and tube colonies (tube roll cultures). By means of the Arabic numerals and the letters any one of the 100 areas can be located and designated for demonstration purposes.

to redness. Singe free exposed end of the tube cotton plug in flame to kill and remove microbes and spores on the outer part of the cotton. The inoculated tubes are then numbered and incubated. In due time the cultural characteristics are noted and the observations entered in a suitable note-book.

Subcultures may also be made in Petri dishes, on potatoes, in tubes con-

taining bouillon broth, blood serum, milk and other media with or without indicators.

C. *Studying Anaërobic Microbes.*—Some microbes have anaërobic tendencies (facultative aërobes) and some are absolutely anaërobic (obligative anaërobes). The deep stab culture will show anaërobic tendencies. If such tendency exists, development will be more active near the bottom of tube (in the line of the stab). The culturing of obligative anaërobes requires special apparatus though the methods are not in any way difficult. The following methods are used:

a. Deep stab culture. This has already been sufficiently explained. It merely indicates possible anaërobic *tendencies.*

b. High-culture methods. Fill the tube of a deep stab culture, shallow stab or streak, with liquid agar or gelatin and incubate in the usual way. The medium to be poured must not be warmer than is absolutely necessary to render it liquid. This brings out possible anaërobic tendencies to a more marked degree than does the simple deep stab culture.

c. Make an Esmarch roll tube culture as follows: Roll a dilution

FIG. 42.—Hanging-drop culture, sectional profile view. These slides can be procured from dealers in microscopical supplies.

gelatin or agar tube culture (1:10, 1:100, 1:1000, etc.) so that all of the medium (5 c.c., 9 c.c. to 10 c.c.) is spread over the inner surface of the tube to within a short distance of the cotton plug. Keep on rolling slowly until the medium has set. Roll on ice, under the tap water, in ice water, holding the tube at the proper slant. When the medium has set, fill in the entire tube with liquefied gelatin or agar; cool, and incubate. Like the other methods described, this will show possible anaërobic tendencies.

d. Various methods are used to either remove the air (vacuum), displace the air, or remove the oxygen from the air. In the so-called Buchner method, potassium hydroxide and pyrogallic acid are used to take up the oxygen of the air. The air in a suitable container may be replaced by hydrogen by means of a Kipp generator. As it is not likely that the pharmacist will have any occasion to employ these methods we shall pass them by with this mere mention. The full description of the methods will be found in any of the larger works on medical bacteriology or in the larger text-books on bacteriological technic.

D. *Microscopical Examination of Microbes.*—The compound microscope is used in examining hanging-drop cultures, water mounts and cover-glass preparations. To make a hanging-drop culture, hollow ground slides

(concave center) are required. Touch a small drop of the culture to be examined on the center of a clean and heat-sterilized cover-glass, by means of a heat-sterilized platinum wire loop. Smear a little plain petrolatum around the rim of the concavity of the slide and invert the cover-glass preparation upon the slide, pressing it gently in place on the petrolatum.

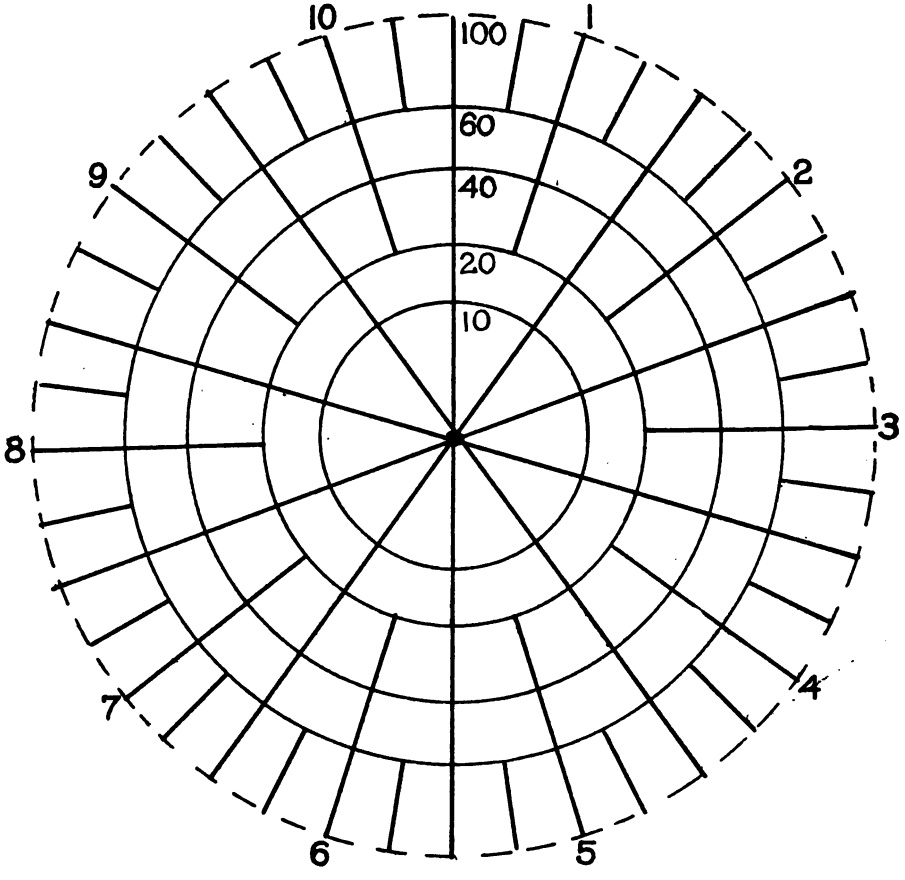


FIG. 43.—Jeffer's circular counting plate for Petri dish cultures. The entire area (100 sq. cm.) is marked off into ten equal sectors of ten-sq. cm. each.

Examine for a period of several hours or longer as may be desired. Cell division, spore formation, etc., can be studied very conveniently. Observations on the effects of temperature and rate of septation may be made. The hanging-block preparation is made by touching the surface of a cube of nutrient agar with the bacteria and then applying this bacterial side against

the cover-glass and mounting like the hanging drop. The bacteria will be found close to the cover-glass.

Bacteria can be examined mounted in water on a slide covered with cover-glass, in order to make observations regarding motility. Of course it is not desirable to examine pathogenic microbes in this manner because of the possibility of infection. In any case, great care should be observed in making the mounts. The slides, covers and needle used must be sterilized, every antiseptic precaution must be observed; and avoid placing an excess of the material on the slide. As soon as the observation is completed (few minutes to half an hour) the mount (slide cover and all) should be placed in a 5 per cent. solution of carbolic acid preparatory to cleaning.

Cover-glass preparations, temporary and permanent, are made as follows:

a. Clean a cover-glass thoroughly, dry it well and heat it. The heating will cause the smear to spread better and to adhere better. The slides to be used must also be clean and dry.

b. By means of the platinum needle, spread a bit of the bacterial growth or culture over the greater portion of the surface of the cover-glass. Add a droplet of water, if desired, to separate the bacteria more. Spread evenly. Do not use too much material, as it will make an unsightly mount.

c. Air-dry the smear preparation. This requires but little time, perhaps a minute or two.

d. Pass the cover-glass preparation through the flame of a Bunsen burner four times. This must not be done too slowly as that will char or burn the microbes, nor yet too quickly, as that would not coagulate the albuminous matter and thus fail to fix the microbes upon the cover-glass. A little experience will soon teach the proper speed. Four seconds, or a little less, is the average time in which to make the four passages through the flame.

e. Place a drop or two of the stain on the fixed smear and allow it to act long enough to stain sufficiently, holding the cover-glass over a flame to warm the preparation. Do not heat it more than 60° to 70° C. On an average the stain will be sufficiently deep in five minutes. Fuchsin requires longer time than does methyl-blue or gentian-violet.

f. Wash off the excess of the stain under a small hydrant stream or by means of a wash bottle, or by moving it about in a dish of water.

g. After washing, the preparation may be examined as a temporary water mount. If it is satisfactory it may be made a permanent mount by turning the cover-glass up again and allowing the water to evaporate and then mounting in Canada balsam with xylene, oil of cloves or some other diluent for Canada balsam. Oil of cloves acts on the stain for which reason xylene, benzene or some other balsam diluent of the coal-tar series is preferable. Special staining methods have already been explained. The above is a

general method which will serve most purposes. It should be kept in mind that the staining process shrinks the microbes somewhat. The ordinary staining methods do not bring out the cilia. The fact that the microbe is motile is evidence that cilia are present, though it cannot be known whether they are unipolar, bipolar or general, single or multiple.

## CHAPTER VI.

## BACTERIA IN THE INDUSTRIES.

A more careful study into the use made of bacteria in the arts and industries will completely dispel the generally prevalent opinions regarding the pernicious nature of bacteria. This erroneous popular conception was the outcome of the earlier activities in the study of disease germs. We know that without the rotting bacteria, higher life, as we know it, would be impossible. Decayed plants and animals mixed with sand constitutes the soil, the ultimate source of all higher life.

### 1. The Function of Bacteria in Agriculture.

The exact relationship of soil bacteria to soil fertility is not generally understood, although it is well known that bacteria are abundantly present in all soils. In fact, soil would be impossible without bacterial action. The number of microbes in one gram of dry soil varies considerably, ranging from about one million to six millions and more. That these minute organisms must perform some important work is almost self-evident. Recent investigations have demonstrated that the fertility of the soil is approximately proportional to the number or quantity of bacteria present.

We recognize what is known as potential fertility and kinetic soil fertility, or, in other words, unavailable and available fertility. By potential or unavailable fertility, we mean the existence in the soil of plant foods which are, so to speak, locked up and cannot be used by plants in the form or chemical combination in which they then exist. By kinetic fertility we mean that condition of the soil in which foods are directly available to the plants growing therein. If all the plant food substances occurring in the soil were directly or kinetically available, the productiveness of the soil would not lessen appreciably for many years, say one hundred years or more. Why then is it necessary to use fertilizers, to rotate crops, to rest the soil, etc., in a few years in order to prevent soil exhaustion? This is simply due to the fact that in a few years the crop plants use up so much of the avaliable food that, unless more is supplied, the crop yield will grow less and less until profitable culture is impossible.

We are familiar in a general way with soil exhaustion and the beneficial influence of soil tilling, of crop rotation, and the use and value of the various fertilizers. It is known that to let crop lands lie fallow for a season or two

renews the productiveness. As already indicated, soil exhaustion means that the crop plants in a few years use up a high percentage of the available foodstuffs. By proper tillage the moisture retaining power of the soil is increased and air is introduced, conditions which are favorable to the development of soil bacteria which have the power of converting a new supply of unavailable plant food into available plant foods. When a fertilizer, as manure or guano, is added to the soil, it is first attacked by myriads of rotting bacteria, which convert some of the insoluble organic manure compounds into soluble compounds, known as peptones and albumoses. These are in turn converted into ammonia by other microbes, and the ammonia is converted into nitric acid by the so-called nitrifying bacteria. The nitric acid at once combines with potash and lime in the soil, forming potassium nitrate and calcium nitrate, in which form these substances are available as plant foods.

Eminent scientists declare that certain bacteria of the intestinal tract are absolutely essential to life. Those bacteria constantly associated with the roots of plants presumably play a very important part in the life history of these plants. The mutually beneficial biological relationships or associations (mutualistic symbiosis) between bacteria and animals and between bacteria and plants are very numerous. In fact the antagonistic (parasitic) or objectionable associations are a decided minority. The recent investigations along this line have revealed some very interesting life conditions, as will be more fully explained in the discussion of industrial bacteria.

In green manuring, microbes and higher fungi cause the starch, sugar, and cellulose of the plants used for this purpose to undergo fermentation; organic acids are liberated which render the insoluble soil phosphates (of calcium) soluble. That is, the insoluble basic phosphates are converted into neutral phosphates, which are soluble. Carbon dioxide, another very important bacterial product, combines with potash to form carbonates, and these in turn act upon the silica in the soil, forming the potash zeolites (hydrates of silica). Certain microbes, lower hyphal fungi and soil algæ, have the power of chemically binding the free nitrogen of the air, thus rendering this abundant element available as plant food.

By means of thorough soil cultivation and the systematic use of fertilizers we simply encourage the development of the particular microbes that will set free or render available the food substances required by the crop plants under cultivation. Agricultural bacteriology is beginning to make practical use of certain plant food forming microbes. Of these the free nitrogen-binding microbes are most promising from the standpoint of practical commercial utility, and have received much attention in recent years. The more important species are: *Rhizobium mutabile, Bacillus ellenbachiensis* Caron, *Azotobacter chroococcum; Bacillus subtilis, Bacillus californiensis*, and

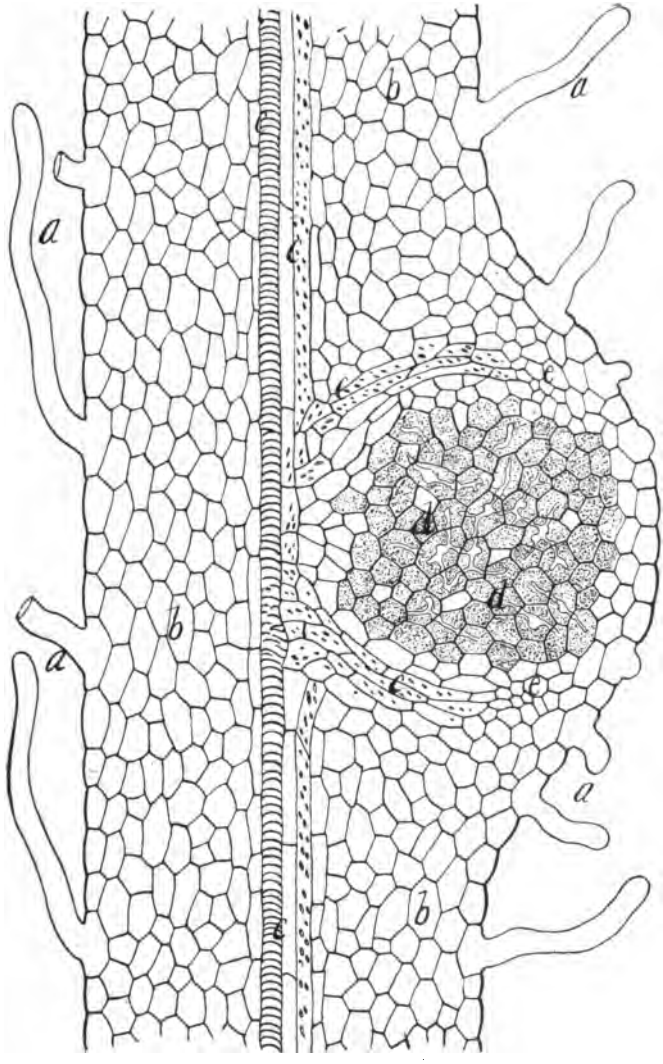


FIG. 44.—Longitudinal section through red clover rootlet, showing tubercle formation due to the root nodule microbe, *Rhizobium mutabile*. The tubercle is only partially developed. *a*, root hairs. These do not develop on the nodule. *b*, the normal root parenchyma. *c*, vascular tissue. *d*, infected area, also showing the infecting strands (*Infectionsfäden*). The cells are filled with bacteria. *e*, apical areas, the growing areas of the tubercle.

a few others. Of these, *Rhizobium mutabile*, the root-nodule bacterium of the Leguminosæ, has received most attention.

The first to suggest a plan for practically utilizing the root nodule bacteria (Rhizobia) and to secure letters patent for the process in Germany and the United States, were Nobbe and Hiltner, of Tharand, Germany. Patent No. 570,876 was granted Nobbe and Hiltner in the United States, November 3, 1896. This patented fertilizer for leguminous plants consisted of pure cultures of the several varieties (or perhaps species) of *R. mutabile*, each species of plant, as bean, pea, clover, alfalfa, etc., having the cultures derived from the root nodules peculiar to it.

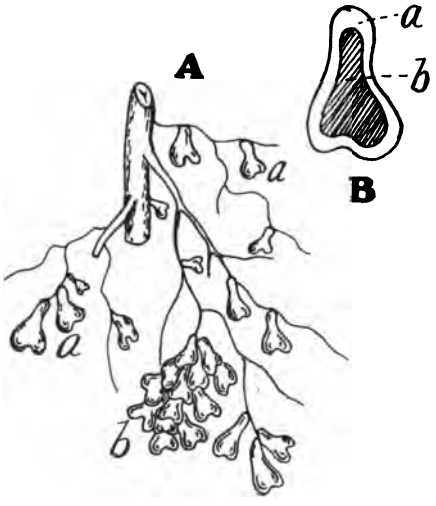


FIG. 45.—Root nodules of sweet clover, somewhat magnified. *A*, rootlets with nodules. *a*, single nodules. *b*, clusters of nodules. These are sometimes very large, consisting of hundreds of nodules, loosely united. *B*, diagram of single nodule. *a*, uninfected area. *b*, infected area.

This commercial preparation was given the name "nitragin," and its efficiency was quite carefully and extensively tested and commented upon by European and American investigators. The consensus of opinion seems to be that it was of doubtful practical utility for agricultural purposes. Some authorities maintained that it was of unquestionable value in virgin soil. In rich and otherwise favorable soil conditions it is of only slight value. It is maintained that nitragin aids very materially in developing and ripening the fruit. As becomes evident from careful consideration, the value of this microbic fertilizer depends upon whether or not it will cause an increased development in the number and size of root tubercles over and above those which would develop without the presence of this artificial aid. If the soil

is already well supplied with rhizobia or root tubercle bacteria, as soil would naturally be if the leguminous plants under consideration had been grown in it for one or more seasons, nitragin would in all probability be of little or no value. In any case, the anticipated results have not been fully realized, and nitragin is withdrawn from the market, and is no longer manufactured.

A second and later improvement in the method of inoculating seeds with root tubercle bacteria (Rhizobia) is given by Hartleb in the specifications forming part of letters patent No. 674,765, granted May 21, 1901, at Washington, D. C. Although not so stated in the specifications, it is evident that

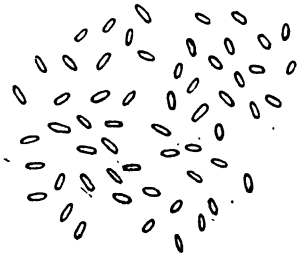

FIG. 46.—Motile forms of *Rhizobium mutabile* as they appear in fresh cultures. They are very small, 1/2 to 2/3 $\mu$ in length.

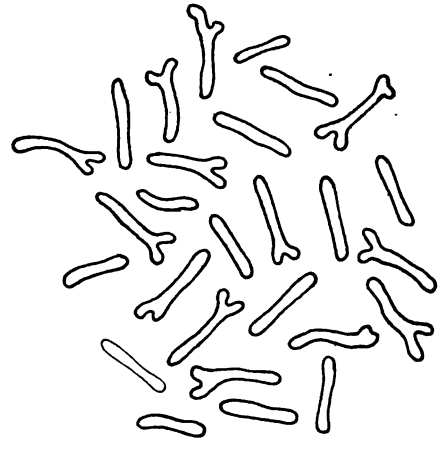

FIG. 47.—Non-motile matured forms of *R. mutabile* as they appear in mature sweet clover root nodules. Most of them show the forked ends. This may be considered the normal form of this organism.

the Hartleb process is a method for applying pure rhizobia cultures to seed of leguminous plants. Whether the method offers any advantages over the method of Nobbe and Hiltner is questionable. In any case it would prove practically advantageous only under the conditions referred to under the discussion of nitragin. Although the method has been freely discussed and experimented upon in Germany, the fertilizer is no longer on the market. There is on the market a third patented germ or microbe soil fertilizer of German origin, known as "alinit." It consists essentially of a pure culture of the soil bacillus known as *Bacillus ellenbachiensis alpha* or *Bacillus ellenbachiensis* Caron. The germ was first brought to the attention of the agriculturists by Caron, a land owner of Germany, who first isolated it and called attention to the fact that it had the power of chemically binding the free nitrogen of the air. The microbe is said to be closely allied to *B. mega-*

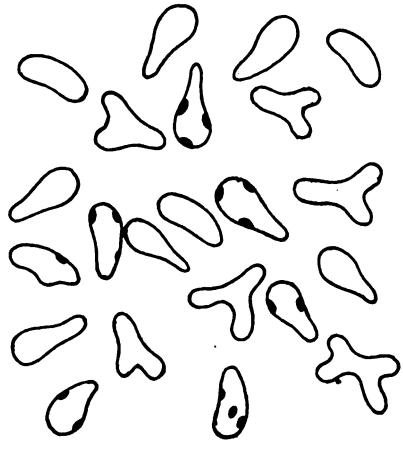

FIG. 48.—*R. mutabile* as it appears in mature nodules of red and white clover root nodules. This may be considered the extreme form variation due to hyper-nutrition.

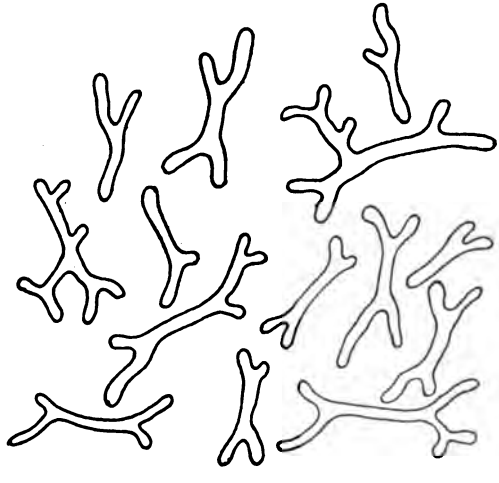

FIG. 49.—*R. mutabile* from the root nodules of *Trifolium heterodon*, showing the extreme form variation due to hyper-growth. The forms shown in Figs. 7, 8 and 9 are simply natural involution forms of the same species due to differences in environment and host relationship. The chromatin bodies found in the hyper-nourished forms (Fig. 48) are probably reserve products.

*therium* and *B. subtilis*. According to some authorities it is especially concerned in assimilating free nitrogen for gramineous plants. If it is true it may prove of great value to grain growers.

The commercial alinit is a dry pulverulent substance of a yellowish-gray color, with about 10 per cent. moisture and 2.5 per cent. nitrogen. It is evidently prepared by mixing spore-bearing pure cultures of the bacillus of Caron, with a base of starch and albumen. It is used to inoculate soil either by spreading it broadcast or by sowing or otherwise planting it with

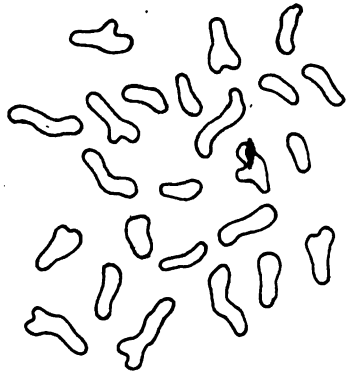

FIG. 50.

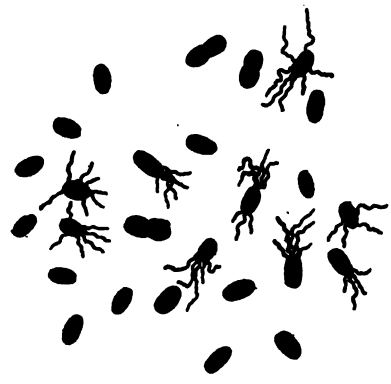

FIG. 51.

FIG. 50.—Involution forms of *R. mutabile* as they occur in artificial culture (beef broth). *R. mutabile* can be cultured quite readily upon a great variety of culture media, showing marked adaptibility to variations in food supply and in environment.

FIG. 51.—*Azotobacter agilis* deeply stained. This organism is actively motile as indicated by the pressure of numerous cilia. The closely related species *A. chroöcoccum* is less actively motile. Both possess the power of free nitrogen assimilation to a high degree, especially when cultured in a nitrogen-free medium. The organisms are large (3 to 6 $\mu$ in diameter) in the comparative sense. *Clostridium pastorianum* is also an active free nitrogen assimilator, but differs from the Azotobacters in that it forms spores, a property which may render it highly valuable in economic agriculture as cultures in the sporulating stage can be kept for a long time while the cultures of non-sporulating bacteria soon die off or lose their potency.

the seed. It is not a nodule or root tubercle-forming organism, and does not enter into intimate symbiotic or biologic relationship with plants. Its work is simply that of binding free nitrogen, forming nitrogenous compounds which enrich the soil, thus increasing the yield of any crop benefited by such compounds.

It is known that there are soil bacteria which are more especially active with certain plants or groups of related plants, and this peculiarity has suggested the possibility of isolating them, artificially increasing their potency and using them commercially for fertilizing purposes. It is also true that not all soil bacteria are beneficent. Under certain conditions, pathogenic and otherwise, harmful microbes are present in great numbers and become

very destructive to crop plants, causing diseases of roots and other plant organs. *Bacillus californiensis*, isolated from sugar beets and from sugar beet soil, appears to promote the growth of sugar beets, particularly the seedlings. The microbic leguminous fertilizer of the Department of Agriculture, Washington, D. C., is a slight modification of the Hiltner method. The microbic cultures are grown in the absence of nitrogen or nitrogenous compound making them nitrogen hungry, thus increasing their potency to produce nodules when brought in association with germinating leguminous plants. The process is patented in the United States, and free samples have been liberally distributed among farmers for test purposes, but the results reported have been rather variable, and as a whole quite unsatisfactory. The indications are, however, that future experiments will clear up the present difficulties, and some of these so-called vest-pocket microbic fertilizers will no doubt prove highly beneficial.

## 2. Bacteria in Milk and in the Dairying Industry.

Bacteria play an important part in modern dairying, and they are destined to play even a more significant part in the near future. Certain microbes

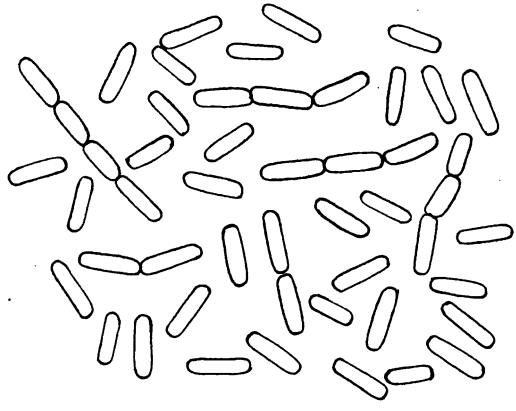

FIG. 52.—Lactic acid bacillus. There is a large group of bacteria, similar in appearance to the lactic acid bacillus, which have the power of forming lactic acid in milk. Some of these are used in pure culture to make the so-called artificial buttermilk. Milk bacteriology is still in its infancy. For so long have we been accustomed to the use of contaminated (filthy) milk that in a recent test made with samples of pure milk and samples of which cow manure was added, 90 per cent. of those who were asked to taste the milks preferred the milk to which the cow manure was added, declaring that it was the only sample which had a "milk flavor."

are active in the ripening of cream, butter and cheese. Formerly it was customary to let nature attend to the inoculation of the cheese, resulting in a rather variable product. Now the up-to-date dairy-man inoculates the

cheese with pure cultures of the kind of microbe producing the desired flavor as Roquefort, Bre, Limburger, etc. In time it will no doubt be possible to produce hitherto unheard-of cheese flavors by means of new species, varieties, and strains of cheese microbes. Cream- and butter-flavor bacteria are also used. The souring of milk is due to the omnipresent but illy defined *Bacillus acidi lactici* and other bacteria. Stringy or ropy milk is due to bacterial infection. Under conditions favorable to the development of the organisms, the ropiness appears within from twelve to twenty-four hours after milking, and becomes so pronounced that the milk can be drawn out in long threads or strings. It is a not uncommon condition of milk in Switzerland, where it is considered specially noxious, but in Holland it has been produced by design for making Edam cheese. Ropiness of milk is caused by a variety of micro-organisms, among them being *Bacillus actinobacter*, *B. lactis viscosus*, *B. gummosus*, etc. The micro-organism used in Holland for the manufacture of the cheese referred to is known as the *Streptococcus hollandicus*. The *Bacillus cyanogenus* causes the milk to become blue without coagulating it or rendering it acid. The *Bacillus butyricus* occurs in milk which it coagulates, also producing butyric acid. It is this microbe which develops the rancidity of butter. There are, however, many different species of microbes which produce butyric acid fermentation.

Freshly drawn milk is not germ-free, even under the most aseptic and sanitary conditions and surroundings. As a rule even the milk in the udder contains some germs, in spite of the fact that milk possesses decidedly bactericidal properties. However, the milk from different animals varies in this regard. The bacterial impurities of freshly drawn milk are traceable to the skin of the cow, the dust and filth about cow stables, the vessel containing the milk, and above all to the hands of the milkers. The milker is often the cause of inoculating the milk with disease germs, as typhoid, colon bacillus, diphtheria, scarlet fever, small-pox, and tuberculosis. The medical journals cite cases of typhoid epidemics traceable to milkers who were "typhoid carriers" without actually suffering from the disease. Cows are very susceptible to tuberculosis, and the milk from tuberculous animals has infected thousands upon thousands of children and many adults.

Since milk is an excellent culture medium for a great variety of germs, it is evident that, under favorable conditions, it may be a fruitful source of infections. Serious epidemics of typhoid fever and of diphtheria have been traceable to and exactly limited to the milk route of a certain dairy-man. Tuberculous infections of the children in a number of families have been traceable to the milk from a single animal. As a rule mixed milk (that is the milk from many animals) is safer than the milk from a single animal, though this is not necessarily always the case. The milk from animals that are free from disease and that are tested regularly (every six months) for tuberculosis,

and that are kept under sanitary conditions, is absolutely safe, provided the containers are clean and the milkers and others in the dairying establishment are free from latent or active communicable disease and are cleanly in their habits. The number of germs in freshly drawn milk varies from 1000 to several millions per c. c., and is directly proportional (within the limits indicated) to the cleanliness and sanitary conditions of the dairying establishment. The bacterial content of milk from the same source is of course higher in warm and hot weather than it is in cold weather, other things being equal. Certain dairying establishments supply what is known as "certified milk," or milk which is certified by the board of health as coming from animals that are regularly tested for tuberculosis and which are kept under the sanitary conditions imposed by the milk commission or by the board of health, furthermore, such milk must be bottled in sterilized bottles which are hermetically sealed and placed on ice at once and kept on ice until delivered to the consumer. There is, however, a lack of uniformity in the regulations governing the supply of certified milk in different communities. The following conditions *should* prevail:

a. All cows should be healthy, that is, free from diseases of *all* kinds. The animals should be tested for tuberculosis every six months. As soon as an animal gives a positive reaction for tuberculosis, it should be removed from the herd and killed. Milk from sick animals (any disease whatever) should not be used.

b. The sanitary conditions and environment of pasture, grazing lands, sheds, stables, etc., should be excellent. The entire water supply should be pure, and all water supplies should be tested chemically and bacteriologically at suitable intervals. All food supply for cows must be wholesome and free from objectionable contaminations.

c. Those employed about the establishment must be free from latent or active disease. They should be tested for tuberculosis, latent typhoid, and should be examined for skin diseases. They must be cleanly in their habits. Before milking, the hands of the milkers and the teats of the animals should be washed with clean warm water and then dried with a clean towel.

d. The containers must be sterilized thoroughly every day, inside and outside. This can be done by thoroughly washing and rinsing in boiling hot water and thoroughly drying, before pouring milk into them.

e. Just as soon as the milk is drawn, it should be bottled (sterilized bottles), bottles capped, hermetically sealed (paraffin), and placed on ice until ice-cold, and delivered at once to the consumer. The bottles should be on ice in delivery, and, even though hermetically sealed, should be kept away from dust and dirt. The bottles should be placed in paper bags so that the driver need not touch them at all. The housewife should take the bottle

from the bag and place it in the ice-chest, cellar, or cooler until the milk is wanted for use.

Such certified milk would, in all probability, contain less than 1000 microbes per c.c., perhaps not more than 200 to 500 per c.c., whereas most of the so-called certified milk found on the market contains from 1000 to 10,000 and more microbes per c.c. The bacterial content of fresh uncertified milk ranges from 20,000 to several millions, although from 20,000 to 50,000 per c.c. is the maximum number allowed by most boards of health. As already stated, milk is an excellent culture medium for bacteria, and under favorable temperature conditions the rate of development is very rapid. In the United States the requirements of the bacteriological standardization of milk are very variable and are rather arbitrarily fixed by the different boards of health that may insist upon any standard at all. In some cities a summer and a winter standard is recognized. 75,000 bacteria per c.c. may be the winter standard, while 100,000 per c.c. is the summer standard. Nearly all boards of health admit that 3,000,000 bacteria per c.c. is the maximum number which may be permissible.

Milk, on standing, should show no dirt deposit. This crude test is a fairly reliable guide as to the sanitary conditions in the dairying establishment and the rules of cleanliness that are observed. It has been shown that the quantity of bacteria in freshly drawn milk is directly proportional to the amount of dirt (sediment) present. A bottle or tumbler full of milk should show no dirt sediment after standing for an hour or more.

Good cows' milk should have from 3.5 to 3.75 per cent. of butter fat. It is marketed in three forms: Full milk having all of the butter fat, half milk or partially skimmed milk, and skimmed milk. Because of the variability of milk which is partially skimmed, it would be wise to withdraw it from the market. When milk is sold without further specification, full or unskimmed milk is understood. It is unlawful to sell skimmed milk as milk, or without designating it as skimmed milk.

In some countries, as Germany for example, the rules and regulations directed against dairies, dairying and the sale of milk, are very far-reaching, and are strictly enforced by the local health authorities. Specific rules are laid down as to what milk may or may not be marketed, how the cows are to be kept, what cattle diseases render the milk unfit for use, how cows and milkers must be prepared for the milking process, etc. The use of preservatives is not permitted, because these substances reduce the digestibility of the milk and because their use encourages lax and careless methods in the dairying establishments.

The bovine disease most to be dreaded is tuberculosis. It is very prevalent among cattle, and the milk from tuberculous cows is a serious menace to the health of those who use it, particularly to susceptible (by inheritance)

children. The most efficient means of safeguarding the public health against this source of infection consists in removing the infected animals from the herd, with a view of disposing of them by slaughter and burial as soon as circumstances will permit. Where this wasteful method has been employed the results have been discouraging, even when the State recompensed the owner in part for the loss of his stock. The government meat inspection regulations admit the use of meat of slightly tuberculous animals for food, for it is declared that under such circumstances the cooking of meat is an effective safeguard against danger.

Testing cows for the presence of latent or undeveloped forms of tuberculosis is simple, safe, and should be rigidly persisted in. Tuberculin is injected into the neck or shoulder region. If tuberculosis exists there will be a rise in temperature (102° to 104° F.), in the course of from eight to eighteen hours. If the disease is far advanced there may be no reaction, in fact, the reaction is then unnecessary as the indications are already sufficiently positive.

The tuberculin used is prepared from glycerinated bouillon in which tubercle bacilli have been grown from six to eight weeks. The bouillon culture is first boiled for two hours to kill all the living organisms. It is then filtered under pressure through a germ-proof earthenware filter to remove the dead bodies of the germs, concentrated by evaporation, a little carbolic acid added, and it is then bottled for distribution. There is no evidence that its use causes an increase in the rapidity of the progress of the disease in animals already affected with tuberculosis, or that it is injurious to them in any other way. It does not even temporarily injure the quality of the milk.

Preservatives, as boric acid, salicylic acid, benzoic acid, sodium benzoate and formalin, are sometimes added to milk to prevent bacterial development. A very small amount of formalin (1:10,000) is sufficient to check the souring of milk. The others are added in larger amounts (1:1000 or more). These additions are not, as a rule, appreciable through the sense of taste or smell and do not in any way modify the appearance of the milk. In some countries milk preservatives are permissible, in others they are not, and in still others they are permitted provided there is a declaration to that effect and the amount does not exceed a definite percentage, as provided by law.

In England, a limited amount of certain preservatives added to milk is permissible, the argument being that it is better to supply preserved milk than milk loaded with germs. This argument has its commendable features. In very large, congested cities like London, New York and Chicago, it is impossible to supply the poor with certified milk or milk which can be kept free from excessive germ development until it is wanted for consumption.

Boiling the milk for twenty minutes kills the germs, but unfortunately the boiling temperature produces certain changes which greatly reduce the food value of the milk, besides the germicidal properties of the milk are destroyed,

so that the bacterial development is afterward even more active than before. Sterilizing at lower temperature (50° to 80° C.), known as pasteurizing, does not interfere with the nutritive qualities of the milk, but destroys the bactericidal properties, as already mentioned. The process is, however, generally recommended by physicians. A simple home method may be carried out as follows (Roger):

Milk is most conveniently pasteurized in the bottles in which it is delivered. To do this use a small pail with a perforated false bottom. An inverted pie tin with a few holes punched in it will answer this purpose Punch a hole through the cap of one of the bottles and insert a thermometer. Set the bottles of milk on the pie tin in the pail and fill the pail with water nearly to the level of the milk. Put the pail on the stove or over a gas flame and heat it until the thermometer in the milk shows not less than 65° C. nor more than 70° C. The bottles should then be removed from the water and allowed to stand from twenty to thirty minutes. The temperature will fall slowly, but may be held more uniformly by covering the bottles with a towel. The punctured cap should be replaced with a new one, or the opening sealed with wax or paraffin, or the bottle may be covered with an inverted cup.

After the milk has been held as directed it should be cooled as quickly and as much as possible by setting in water. To avoid danger of breaking the bottle by a too sudden change of temperature, this water should be warm at first. Replace the warm water slowly with cold water. After cooling, milk should in all cases be kept at the lowest available temperature.

It should be remembered that pasteurization does not destroy all bacteria in milk, and after pasteurization it should be kept cold and used as soon as possible.

Rosenau sums up the *pros* and *cons* of milk pasteurization as follows:

*Advantages.*—The advantage of pasteurization is that it is a cheap and effective means of preventing the transmission of infectious diseases such as tuberculosis, typhoid fever, diphtheria, scarlet fever, etc., commonly spread by milk.

*Disadvantages.—a.* Pasteurization promotes carelessness on the farm and dairy, etc. (This may be controlled by proper regulations, inspections and laboratory examinations.)

*b.* Pasteurization renders milk less digestible. (While it is generally conceded that boiled milk commonly induces constipation, the majority of the evidence plainly indicates that pasteurization has little, if any, effect on the digestibility of the milk.)

*c.* Pasteurized milk favors the production of rickets and scurvy. (There is no proof to this effect and authorities agree that the danger is slight; and, further, that it may readily be obviated.)

*d.* By destroying the non-spore-bearing bacteria, pasteurization sometimes allows toxic organisms to grow and produce serious poisons in the milk. (On the other hand, these same poisons are more frequently produced in milk that has not been pasteurized, and thus danger may be obviated in pasteurized milk by cooling it quickly, keeping it cold and shortening the time for distribution.)

*e.* Pasteurization is inefficient as a preservative; the milk keeps only twelve to twenty-four hours longer than otherwise. (This is really no disadvantage, for the quicker bad milk sours, the better.)

*f.* Pasteurization injures the taste of the milk. (This is not so, if properly done.)

*g.* Pasteurization increases the cost of the milk. (True, but it is the cheapest safeguard, and the expense of pasteurization is offset by the keeping quality of the milk.)

Rosenau has made extensive tests to determine the thermal death-point of those pathogenic microbes most commonly found in milk. His conclusions are summarized as follows:

Milk heated to 60° C. and maintained at that temperature for two minutes will kill the typhoid bacillus. The great majority of these organisms are killed by the time the temperature reaches 59° C., and few survive to 60° C.

The diphtheria bacillus succumbs at comparatively low temperatures. Oftentimes it fails to grow after heating to 55° C. Some occasionally survive until the milk reaches 60° C.

The cholera vibrio is similar to the diphtheria bacillus regarding its thermal death-point. It is usually destroyed when the milk reaches 55° C.; only once did it survive to 60° C. under the conditions of the experiments.

The dysentery bacillus is somewhat more resistant to heat than the typhoid bacillus. It sometimes withstands heating at 60° C. for five minutes. All are killed at 60° C. for ten minutes.

So far as can be judged from the meager evidence at hand, 60° C. for twenty minutes is more than sufficient to destroy the infective principle of Malta fever in milk. *M. melitensis* is not killed at 55° C. for a short time; the great majority die at 58° C., and at 60° C. all are killed.

Milk heated to 60° C. and maintained at that temperature for twenty minutes may, therefore, be considered safe so far as conveying infection with the micro-organisms tested is concerned.

Evaporated, condensed and dry milk are found upon the market and are extensively used. Sugar is frequently added as a preservative. In making condensed milk, it is evaporated in large pans until it assumes a creamy consistency. Dry milk is prepared by spraying the milk on revolving hot cylinders. The thin film of milk is evaporated to dryness in a moment,

and in that state is scraped from the cylinders. Dry milk is a common ingredient of baby foods and invalid foods, and is also very extensively used in the manufacture of chocolate creams. The condensed and dry milks do not keep long in spite of the greatest care in manufacture. The containers and milk must be thoroughly sterilized or pasteurized, and the cans must not be opened until ready for use. Such preservatives as salicylic and boric acid are sometimes added to condensed milk.

It is known that sweet cream yields a very insipid, flavorless butter, whereas cream which has "soured" for a few days yields a pleasant tasting and pleasingly flavored butter, provided the desirable species or variety of bacteria are present. If the souring is continued too long the flavor may be hopelessly vitiated. In the past it was customary to add a small amount of old cream, having a desirable flavor, to a new lot of cream. This mother cream was designated the "starter." It contained the desirable cream-ripening bacteria, mostly of the lactic acid variety. These old-time natural starters are now largely replaced by starters, prepared in the laboratory consisting of pure cultures of certain breeds or varieties of cream flavor, producing germs of the lactic acid group. A proper regulation of the temperature is very important in the ripening of cream (60° to 75° F.). It is also necessary to pasteurize the cream before adding the bacterial starter in order to prevent the development of microbes which might interfere with the proper development of the starter microbes. Naturally the use of clean, sterilized utensils and uniformity of methods are all-important, in order that uniform results may be obtained.

Cheese flavors are also due to bacterial action, but not wholly so, as many of the higher fungi, as species of *Penicillium* (Camembert Penicillium) and of *Oidium* (*O. lactis*) also play a very important part as flavor producers. The Roquefort cheese owes its characteristic flavor, in part at least, to a variety or form of *Penicillium glaucum*. The qualities and properties of some Swiss and soft Belgian cheeses are largely due to *Oidium lactis*. The ripening of hard cheeses (Cheddar, Edam, American, some Swiss varieties, and others) is due exclusively to bacterial action. Cream, butter and cheese are very prone to the attacks of objectionable bacteria and moulds which cause very unpleasant flavors and bitter taste.

It must also be borne in mind that cream, cheese and butter may carry disease germs. Tubercle bacilli have been reported in these food articles, but it has not been demonstrated that they are frequently present. Typhoid infections have been traced to the use of cream, but no case of typhoid fever has ever been definitely traced to eating butter or cheese. Of course, these articles may become infected after manufacture and thus become a possible means of spreading disease.

### 3. The Lactic Acid Microbe and Kefir Preparation.

Within recent years the subject of intestinal digestion and the relationship of intestinal microbes to digestion and longevity has received much attention. Metchnikoff declares that the early senile cell changes in the body are due to the repeated or chronic autointoxications brought about by certain noxious intestinal ferments of bacterial origin which are absorbed into the circulation. Some of these bacteria, especially those found in the small intestines, are beneficial, secreting enzymes which aid digestion, but the enormous quantity of microbes active in the lower large intestine are for the most part injurious, producing putrefactive changes, liberating toxins which when absorbed into the system in sufficient quantity produce the symptoms of ptomaine poisoning.

In order to combat these objectionable bacterial activities, it is necessary to regulate the bacterial development in the large intestine. Lactic acid has long been known as an efficient remedy in the treatment of various intestinal disorders. It is known that the poor of certain European countries who live largely on potatoes and clabbered or thick milk are notably free from intestinal disorders and are remarkably long-lived. It is known that pickles, sauerkraut and sour milk are excellent bowel regulators, in spite of the fact that these foods, the former two in particular, are well-nigh indigestible and have little food value. The Arabians have long used koumys as a healthful, life-prolonging article of diet. To this class of foods also belongs the Bulgarian yoghurt and the Egyptian raib.

The ferments of koumys, kefir, yoghurt and raib resemble each other in that they are mixed, consisting of several lactic-acid microbes or organisms and yeast organisms. These foods or drinks therefore contain lactic acid and a small amount of alcohol.

As soon as it was determined experimentally that the beneficent action of sour milk, thick or clabbered milk and the above-named special preparations was largely due to the lactic acid formed by specific microbes, efforts were made to isolate these organisms in pure culture and to induce them to act in sterile or pure milk. This has been done, and there are now upon the European and American market several patented preparations consisting of the lactic acid bacillus.

Our knowledge of the relative importance of the several organisms which are said to produce the fermentative changes in the milk is as yet incomplete. Bacteriologists have thus far not succeeded in disclosing all of nature's secret processes involved. It is supposed that the microbe of Bulgarian sour milk, the *Bacillus bulgaricus*, is the most vigorous and active of all organisms concerned in the lactic-acid fermentation of milk.

It is not definitely determined whether or not the fermentations of milk

induced by the mixed and often filthy "yeasts" employed in making koumys, kefir, yoghurt, matzoon and other similar fermented milk foods, are superior or inferior to those of lactone and other pure culture milk ferments. It is, however, very evident that the marketed preparations in tablet form give very satisfactory results, as used by pharmacists and in the home. Full directions for using the tablets are found on every package. As is naturally to be supposed, these tablets deteriorate in a comparatively short time and all reliable manufacturers place the age-limit on each package.

Pharmacists can prepare a marketable kefir ferment powder from milk activated by kefir, provided care is observed to guard against outside infection in the several steps of procedure. The following is the method of preparing a kefir powder:

A. *Securing the Kefir.*—The kefir known as kefir grains or kefir seeds may be secured from the large dealers in drugs in New York City or in other large Eastern port cities. The kefir is a solid of a tough gelatinous consistency, brittle when dry, of grayish-yellow color. It is a conglomeration of various organisms, as *Dispora caucasica*, several species of other microbes, a yeast organism, and other undetermined organisms.

B. *Washing the Kefir.*—Place two or three drams of the kefir in a mixture of equal parts of milk and water, enough to cover the kefir. Allow to stand for four hours, decant off the liquid and renew at intervals of about one hour. Repeat this four or five times at a temperature of about 82° F.

This process serves a cleansing purpose and initiates the fermentative change. The amount used will depend upon the quantity of powder to be made.

C. *Preparing the New Kefir.*—Wrap the washed and softened kefir in a piece of sterilized gauze and place it in one quart of pasteurized milk. Keep at a temperature of 82° F. Allow to stand for from twelve to fifteen hours, until the milk is curdled.

D. *Skimming and Draining the Kefir.*—Remove the cream and drain the curd (kefir) in sterilized gauze until quite dry.

E. *Drying.*—Add (to the drained kefirized curd) an equal weight of sugar of milk, mix, and spread thinly upon sterilized gauze or upon a sterile glass plate and dry in a current of sterile warm air (80° F.)

F. *Powdering.*—Powder the dried mass gently and put up in dry, sterile, one-ounce, wide-mouthed vials, closed with sterilized corks.

G. *Directions for Use.*—Upon the bottles place the following directions for using the powder thus prepared: "Dilute one quart of milk with one-half pint of water, add a pinch of salt and one level teaspoonful of the powder. Set aside for twelve to fifteen hours at a temperature of 85° F., shaking frequently. Use at once or keep on ice."

There are, of course, no conveniences for regulating the temperature in

the average household, and the action of the powder must take place at the ordinary temperature of the home. Thus the time required to curdle the milk will vary. The powder should be kept in a cool or cold, dry place. Of course, a small amount of kefirized milk can be used to curdle any quantity of fresh milk without using any of the powder.

The pharmacist should test the kefir which he is about to use in preparing the powder, in order to be certain that it is active in curdling milk. Likewise should he test the powder prepared from it.

The kefir powder above described is similar to, although not identical with, certain microbic lactic-acid ferments found on the market, as the lactone tablets, bacillary tablets, yoghurt tablets, fermenlactyl, lacto-bacilline and others. These are prepared from pure cultures of species of lactic-acid bacilli, dried and formed into tablets with some pulverulent (starch, milk, sugar) base, ready for use. The milk (in quart bottles) is first pasteurized, a pinch of salt is added and two or three tablets are crushed and mixed with the milk. In a day or so the milk is transformed into an acidulous drink, resembling buttermilk somewhat in flavor, though it is not buttermilk, as is generally supposed.

These tablets have gained in favor within recent years. They deteriorate in time, as already stated, and the time-limit is stamped on each container. Like the kefir, they act more quickly at a temperature of about 25° C.

As may be readily understood, kefir, lactone, etc., will not produce the characteristic changes in milk to which preservatives have been added; in fact, the failure to produce fermentation is an indication that preservatives are present.

### 4. Microbic Pest Exterminators.

Attempts have been made from time to time to exterminate certain animal pests by inoculating them with some fatal contagious disease of microbic origin. Experiments along this line have been carried on for some time, ever since the causative relationship of microbes and disease was fully established; but it is only within recent years that extensive practical application was made of the use of a few microbic pest exterminators. One of the first to be used with some success was the chintz-bug exterminator. The chintz-bug (*Blissus leucopteris*, also called chinch-bug, chink-bug) was a very destructive corn (*Zea mays*) pest of the Central States (Illinois, Kansas, Nebraska, Iowa), causing great damage to crops during certain very dry seasons. Extensive experiments carried on at the University of Illinois and also at the University of Minnesota (Departments of Agriculture) led to the discovery of a microbic disease of this pest which was quickly fatal and which spread very rapidly. The insects, in cages, were inoculated with pure cultures of the pathogenic microbes, and insects in the diseased condition

were sent to the farmers with instructions how to scatter them through an infested corn-field. The results were in some instances very satisfactory, and again without appreciable effects. The trouble in the use of this exterminator lay in the fact that the climatic conditions (rainy, damp weather) essential to the spreading of the disease did not generally prevail, and as soon as the climatic conditions were favorable inoculation became unnecessary, as the disease developed without artificial aid and effectually checked further ravages.

Rabbits are one of the very annoying field pests of Australia, and attempts have been made to exterminate them by means of pure cultures of microbes capable of developing a fatal infectious disease among these animals, but the results were quite unsatisfactory.

More recently there have been placed on the market quite an array of mice and rat exterminators of microbic origin under various trade names as ratin, rat virus, azoa, rattite, Danysz virus and mouratus. These preparations consist of pure cultures of bacilli pathogenic to rats and mice, as the *Bacillus murisepticus* and *Bacillus typhimurium*, mixed with some inert base, as corn-meal, oat-meal, etc., forming a coarse powder. Some preparations are in liquid form. They are used by mixing the powder or liquid with moist corn-meal or other food material relished by these animals, and spreading it near their haunts and runs. Fortunately, these substances are harmless to man and animals other than mice and rats. These microbic rat and mice exterminators have thus far proven to be rather unsatisfactory. They have undoubtedly given excellent results in some instances, and again they have been absolute failures. The tests made by the University of California, and by Dr. Rupert Blue in his famous plague-rat extermination in San Francisco, have given almost wholly negative results. A microbic squirrel exterminator ("squirrelin") has proven entirely unsatisfactory.

When we consider how difficult it is to *prevent* fatal epidemics, it certainly does seem reasonable to suppose that it should be a comparatively easy matter to find ways and means for *disseminating* fatal epidemics, but so far the commercial attempts made in that direction have proven rather discouraging. Further carefully conducted experiments along this line are necessary. It is known that the ravages of certain pests are sometimes suddenly checked by the natural invasion of some pathogenic organisms. This is frequently observed among plant lice (Aphis) and other insect enemies of plants.

## 5. Bacteria in the Tanning Industry.

The object in tanning leather is to protect it against decomposition and to render it pliable. The various animal hides before reaching the tannery are preserved by drying and salting. At the tannery the hides are treated as follows:

A. *Removing the Hair—Depilation.*—This is done by means of chemicals, as lime or sodium sulphite, or through the agency of rotting bacteria, as *Bacillus vulgaris* (*Proteus*) and others. Just which of several species of rotting bacteria is most active in this process has not been definitely determined.

B. *Drenching or Bating.*—After the hair has been removed, the hides are macerated in an aqueous solution of the excrement or dung of pigeons, hens and dogs. These substances set up a lactic acid fermentation due to the microbes contained therein. The active organisms have not been isolated as yet; *Bacillus gasoformans* and *B. erodiens* are perhaps active, but there are also present many yeasts, moulds and other organisms which may have their special effects.

The first part of this process, known as "bating," is initiated by bird dung; the second process, known as "puring," is due to the action of dog dung. Attempts have been made to use pure cultures of the active microbes to supplant these filth substances, but so far these efforts have not proven wholly successful.

C. *Tanning.*—The bated hides are next treated in the tan pit (coarse skins) or in bark liquor (soft thin skins), where the souring process takes place. This process is also due to bacterial activity. Our knowledge of the action which takes place and of the bacteria involved is very incomplete.

Bacteria are important factors in siloing; in curing tobacco, tea and cacao. The flavor of different brands of tobacco is due to different bacteria, and attempts have been made to isolate those producing desirable flavors and to use them in pure culture. It is highly probable that the bouquet of old wines is due to bacterial action. These are, however, matters which require further study. Rotting bacteria are active in paper-making. In the maceration process certain bacteria feed upon and decompose the less resisting vegetable cell-walls, as those of the parenchymatous tissues, the epidermal tissue, etc., leaving the more resisting fibrous lignified tissues as bast and wood fibers. The pulp is then poured on sieves and the rotted or digested portions washed out.

Bacteria are now practically employed in the purification of sewage. This is done in what are known as "contact beds," in which the environment is made favorable to rapid development of those non-pathogenic rotting bacteria which disintegrate the organic substances and at the same time prevent the development of the pathogenic or otherwise objectionable microbes. It is highly probable that this method may be applied to the purification of streams and other large bodies of water.

The possibilities in the practical utilization of bacteria in the arts and industries are promising, and it may confidently be expected that wonderful innovations along this line will be made in the very near future.

## 6. Cider-making.

Acetic-acid fermentation in wine cider and other fermented alcoholic substances is initiated by the *Mycoderma aceti*, collectively known as "mother of vinegar." This is no doubt a mixed growth, representing several species or varieties of acetic-acid forming organisms. While it is true that nature invariably inoculates the substances named, resulting in the production of vinegar, it is customary to use the top skin or pellicle (mother of vinegar) on vinegar already formed, adding it to new wine or cider in order to hasten the fermentation. As stated, this is not a pure culture representing a single species. In fact, the tests with what were pure species have proven unsatisfactory. The vinegar organisms require an abundance of oxygen. To supply the necessary oxygen (of the air) it is customary to have the fermentation barrels or casks only about two-thirds or three-fourths full and to leave the bunghole open (generally with a plug of cotton). In Germany a quickened method is much in vogue. The wine or cider is allowed to trickle slowly through a cask filled with wood shavings which are moistened with old vinegar. The wood shavings offer a maximum surface exposure and fermentation is as a result very much hastened.

Occasionally the vinegar loses its acidity. This is due to the invasion of a bacillus (*B. xylenum*) which, in the presence of oxygen, splits up the acetic acid into other compounds. This change can be prevented by excluding air from the containers. Vinegar should contain from 4 to 4.5 per cent. of acetic acid (the legal standard).

## CHAPTER VII.

## IMMUNITY AND IMMUNIZING AGENTS.

We speak of immunity from and susceptibility to disease, indicating thereby a difference in individuals regarding their responsive behavior to the factors, forces or influences which may cause or prevent disease manifestations. The subject is one of intense interest in the light of modern biological and bacteriological investigations. It has within recent years received more attention from biologists, physiologists and bacteriologists than any other branch of science and some of the results obtained are in many respects marvelous. The discoveries thus far made are the mere beginnings of future preventive medicine, which will make it possible to establish a system of medical practice of which the chief aim will be to prevent rather than to cure disease.

Several kinds of immunity are recognized which may be tabulated as follows:

- Immunity
  - Inherited
    - Racial (Phylogenetic) — As observed in the different orders, families, genera and species of the animal kingdom.
    - Individual
      - As observed in different individuals of the same species or variety. (Ontogenetic.)
      - As observed in the sexes of the same species or variety.
  - Induced
    - Active
      - Natural — Due to naturally induced infections with disease which produce immunity to subsequent attacks, as diseases of childhood, acclimatization, etc.
      - Artificial — Due to use of modified toxins, bacterins, and direct inoculation with disease germs.
    - Passive—Use of antitoxins and other disease preventives.

It has been known for a long time that when a number of individuals of the same species are exposed to the same bacterial infection, some escape the infection while others do not. That which prevented the development of the bacterial disease or which neutralized the toxic products or which killed the disease organisms thus preventing the disease manifestations, constitutes immunity.

It may be assumed that the members of the various subdivisions of the animal kingdom have in the course of their phylogenetic or evolutional development acquired certain properties of cells, cell-contents, tissues and organs, which enable them to resist certain harmful bacterial invasions, as well as the injurious effects of other noxious influences and substances. For example, the typhoid fever germ is harmless to the oyster and other lower as well as most higher animals, but it is very injurious to man. The vegetable alkaloids are very toxic to man and most other vertebrates, whereas they are harmless to the protozoa and other low forms of animal life. As is known drug parasites feed with impunity upon the most potent vegetable drugs. The carnivora are less liable to bacterial infection than the herbivora. Closely related species sometimes display remarkable immunity differences. For example, field mice are very susceptible to glanders, whereas the common house mouse is almost wholly immune. Jersey cows are less liable to tuberculosis than Holsteins. The Yorkshire breed of swine is less liable to the attacks of hog erysipelas than are other breeds. Man is especially susceptible to malaria, cholera and typhoid fever. Man, cattle and apes are very susceptible to infection by the tubercle bacillus, whereas the wild carnivora are quite exempt. Anthrax may attack man, cattle, sheep and guinea-pigs, whereas birds, rats, cats and dogs are free from such attack. The Caucasian race is less liable to small-pox, tuberculosis and syphilis than is the Negro race. On the other hand, the Negro is more immune to yellow fever than is the white man.

Immunity is, however, very largely relative. The wild carnivora are quite free from disease whereas in prolonged captivity they may fall prey to several diseases, notably tuberculosis. Toxic substances, noxious gases, lack of food, poor food, cold, excessive heat, fatigue, over-exertion, inclement weather, etc., are factors which may lessen the natural immunity to the several infections to which the animal may be exposed. For example, no race of mankind is possessed of absolute immunity to any human disease. Such immunity differences as are observed are due to differences in the opportunities for infection, differences in habit, in occupation, etc.

The modern explanations regarding the mechanism of immunity are extremely interesting and a work on pharmaceutical bacteriology would certainly be incomplete without a brief summary of the discoveries to date.

In 1890 Behring and Kitasato found that the cell-free blood (serum) of rabbits and of mice which had been artificially immunized against tetanus, neutralized or destroyed the toxic substances of the tetanus bacillus. To this substance they gave the name antitoxin. This was an epoch-making discovery. It led to the finding of other antitoxins or antibodies which are now used in the treatment of disease as will be more fully explained in a subsequent chapter. Antitoxins, like the toxins, possess many of the characters of albuminoids, are quite readily decomposed and are incapable of isolation from the blood or from the tissue cells. Never having been obtained in purity nothing is known regarding their physical appearance. They are readily destroyed at comparatively low temperatures (65° to 75° C.) and by exposure to light and air. They are very sensitive to acids and are best preserved by evaporating the blood sera in which they are contained to dryness in a vacuum at a low temperature and storing in a vacuum, at a low temperature, away from light and in a dry place. Experimentally it has been demonstrated that the antitoxins are intimately combined with the globulins of the blood. This discovery led to the manufacture of concentrated antitoxins by precipitating the globulins with ammonium sulphate, magnesium sulphate and other salts. Remarkably enough, reactions have been observed which would indicate that antitoxin is not a proteid substance; for example, it is not destroyed (digested) by trypsin.

It has furthermore been found that variably small amounts of antitoxins exist in normal blood; that is, in the blood of animals that have not been naturally or artificially immunized, and also in still lesser amounts in the milk of normal animals. As to the origin of the antitoxins the physiologic evidence points to their formation in the body cells rather than in the blood serum.

Another important discovery was that normal blood could actively destroy (lake) bacteria, and in common with antitoxins this bactericidal property was found to be specific. That is, serum found to be quite destructive to the typhoid bacillus is not destructive to the cholera bacillus. These germ destroying or bactericidal substances are designated lysins. Ehrlich has discovered that there are in fact three distinct blood lysins; namely, cytolysin, a substance which is capable of destroying (laking) body cells; hemolysin, which is capable of destroying red blood-corpuscles; and bacterolysin as already explained. By injecting tissue cells, as those of kidney or of some other organ, into an animal, there are developed in the blood of the inoculated animal lysins which will dissolve kidney cells or other organ cells used. If the blood of a bird or other animal is injected into an animal of a different species, hemolysins will appear in the blood of the animal thus injected. This hemolysin is specific, as it will only dissolve or destroy the hemoglobin in the blood of the kind of animal of which the blood was used

for injecting. An animal inoculated with the typhoid bacillus will produce a blood lysin which destroys the typhoid bacillus. Lytic sera become inactive when heated to 55° C. for one-half hour and such sera are said to be inactivated. However, if normal serum is added to the inactivated serum the bactericidal power is fully restored. The bactericidal power of the serum can be greatly increased by the use of highly virulent bacterial cultures, thus producing a serum of high potency. In actual practice, as in the manufacture of bactericidal sera for the prevention and cure of disease, the animal (as horse) is first inoculated with attenuated cultures, then with normally virulent cultures and finally with hyper-virulent cultures of the specific pathogenic microbe. Such sera act by destroying the disease-producing bacteria, but they have no effect upon the toxins produced by the bacteria, thus showing that they are entirely distinct from the antitoxins.

The eminent bacteriologist Metchnikoff made the very interesting discovery that the white blood-corpuscles (leucocytes) had the power of feeding upon and digesting bacteria with which they came in contact. That is the white blood-corpuscles, called phagocytes, act as the defenders of the body against bacterial invasion. This observation by Metchnikoff, fully verified by others, is generally known as the phagocyte theory and the phenomenon is designated phagocytosis. The principle involved in phagocytic activity is well illustrated in the lesser local injuries, as cuts, bruises, abrasions, etc. Normally such injuries are always infected by various germs of the environment, as the several varieties of pus microbes. These invading microbes at once begin their attack upon the tissue cells and blood-corpuscles. The leucocytes which are present begin to feed upon the rapidly multiplying pus organisms but for a time, as a rule, the latter have the upper hand and as a result there is perceptible pus formation ("the laudable pus" of older writers) represented by dead leucocytes gorged with microbes. As the inflammatory reaction becomes more marked, indicated by redness and swelling of the tissues immediately about the injury; increased numbers of leucocytes (phagocytes) are brought to the scene of action and gradually they gain control until finally the invading microbes are all destroyed, thus permitting a rapid and unhindered restoring of tissue cells, recognized as the healing process. This phagocytic action is entirely distinct from the action of antitoxins and lysins, and the three are potent factors in immunity.

The investigations of Metchnikoff and Leishman on phagocytosis paved the way for the discovery of opsonins by Wright. It was noticed that the phagocytic activity was influenced by conditions to be found outside of the leucocytes themselves. Metchnikoff held that the principal part is played by substances found in the serum and in the tissue cells to which he gave the name "stimulins." The purpose of these substances in the tissue fluids

have not yet been satisfactorily demonstrated, but Metchnikoff considers their function to be that of acting upon the phagocytes in such a manner as to stimulate them to perform phagocytosis. Wright, Hektoen, Neufeld and others have demonstrated beyond doubt, the presence in the blood of substances which act upon the infecting bacteria and get them ready for the completion of their destruction by the phagocytes. To these bodies he has given the name "opsonins" (Latin, *opsono*, I prepare for). That opsonins are not formed in the blood is certain. Experimental evidence seems to prove that they are products of muscular or subcutaneous cellular activity. It is probable that the actual formation of opsonin occurs in the muscle tissues and passes thence to the blood. Wright has demonstrated more or less satisfactorily the presence of opsonins in the blood of animals and humans and by a special technic has measured the relative amount. This measurement is a ratio of the activity of the phagocytes in normal blood and of that in disease, before and after stimulation, determined by the number of bacteria that a single phagocyte will ingest—the so-called opsonic index. This index or ratio is made intelligible by decimal figures representing the number of bacteria which the average phagocyte will take up. We may assume that one phagocyte in normal blood will ingest an average of 10 bacteria, represented in the index by the figures 1.0, but in disease (chronic) the phagocytes may only take up an average of 3, 6, or other numbers, represented by the figures 0.3, 0.6, etc. After stimulation the phagocytes may take up 15, 25, or even numbers of bacteria represented in the index by the figures 1.5, 2.5, etc.

Taking the opsonic index of an individual's blood calls for considerable delicate technic. In brief, it is performed by mixing together equal volume quantities (measured in a capillary tube) of blood serum and an emulsion of bacteria and incubating for 15 minutes at 37.5° C. Then making a thin smear of the mixture on a microscope slide, drying and staining, and counting the number of bacteria enclosed in each white blood-corpuscle (50 to 200 cells counted) and striking an average. This average is the index stated by a decimal figure. The index thus obtained indicates the relative phagocytic power of the individual's blood tested, whether below or above the normal.

The opsonic index taken in the various chronic forms of bacterial infections is invariably below normal and shows that the phagocytic power is low, and it seems to prove that the chronicity is due to the abnormal phagocytosis. The injection of several millions of devitalized bacteria of the kind causing the infection, induces the formation of the specific opsonin, arouses the phagocytic activity and corrects the pathologic condition. The opsonic method of treatment has been extensively tested through the use of specifically active bacterial suspensions (vaccines, bacterins or

opsonogens) which in some instances have given excellent results. It has also been found that substances other than opsonins may increase phagocytosis, as for example, nucleinic acid and collargol.

From the foregoing it becomes evident that immunity from disease depends upon the presence in the body of antitoxins, bacterolysins, and the

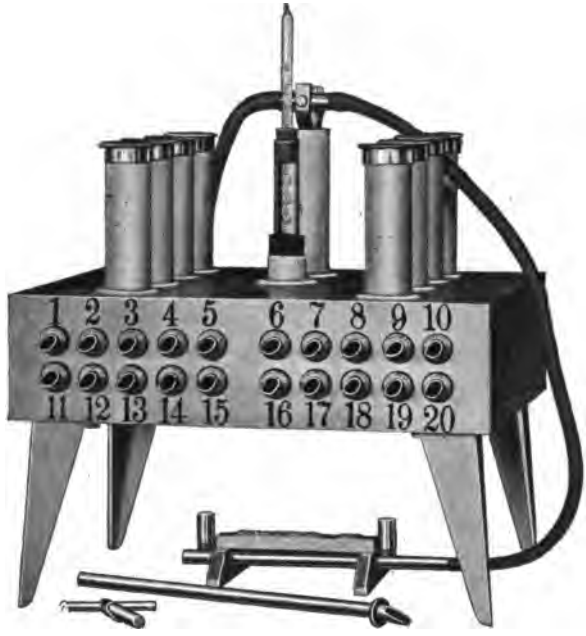


FIG. 53.—Opsonic Incubator. The determination of the Opsonic Index has become so important that these incubators have been made to meet the demands for an apparatus in which twenty pipettes can be incubated at one time, and so that any tube may be examined during the progress of the experiment without changing the temperature of the others. There are twenty tubes for opsonic pipettes and an extra tube. The tubes may be easily removed when desired by means of a key which accompanies the incubator. On top there are eight tubes, 22 mm. in diameter, for test-tubes. Each is provided with a nickel-plated cap. The incubator is supplied with thermometer, thermo-regulator, and a two-flame burner, with wire guard.

opsonins which induce phagocytosis. It is furthermore possible to increase the activity of these agents artificially. All three agents are specific in nature as already stated. Ehrlich has attempted to explain the phenomena of immunity according to his receptor or side chain theory (Seitenkettentheorie). This theory, which is rather complex and highly technical, was first used to explain cell metabolism. Hinman's version of the side chain theory is very simple and we give it as follows: As applied to immunity the basis of the theory is the conception of the duplex nature of antigens.

An antigen is a toxin, of bacterial or other origin, which has the power when introduced into the body, of inducing the formation of specific antibodies. Not all toxins or poisons have this power. For example, strychnin and the toxin of tetanus produce similar physiologic effects, but only the latter is capable of producing an antibody. Ehrlich explains this difference by assuming that strychnin and most other vegetable poisons enter into a loose combination with the cell plasm, analogous to an aniline dye which can be readily dissolved out again; whereas the toxin is firmly bound to the cell, representing in a measure a toxic food-stuff in chemical

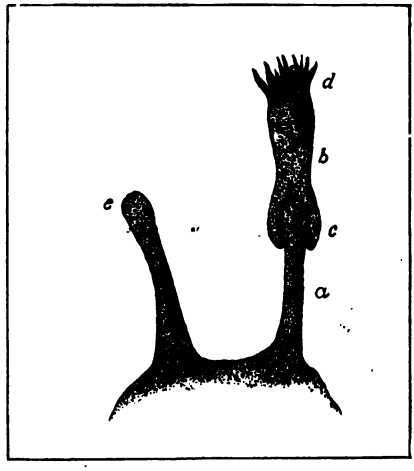


FIG. 54.—Illustrating cell receptors of the first order. A cell receptor (*a*) uniting with the haptophore (*c*) of the toxin molecule or antigen. The toxin molecule or antigen consists of the haptophore and the toxophore. The toxophore produces the toxic effects upon the cell. *e* is the haptophore of the cell receptor which has the power of combining with the toxin molecule thus neutralizing its possible toxic effects. Free-cell receptors constitute the antibodies, and are ever ready to combine with antigens or toxins, should any be present. Cell receptors and antigen bodies are specific in action. The haptophore of the diphtheria cell receptor does not fit the haptophore of tetanus, for example. Each antigen or toxin reacts with the antibodies fitted to it. (*Journal of the American Medical Association*, 1905, p. 955.)

union with and assimilated by the cell. The atomic combination of the toxin antigen, which represents this chemical union is designated the haptophore group, while the atomic combination of the cell-plasm with which the haptophore group unites is called the cell receptor group. The haptophore group is distinct from the atomic group which produces the toxic or pathologic effects, designated as the toxophore group. These two groups of the antigen (toxin), namely, the haptophore group and the toxophore group, act independently of each other and possess different properties. The toxophore group is easily

destroyed by heat (60° to 65° C.) while the haptophore group is not destroyed, retaining the power of combining with the receptor group of the living cell. The toxophore group is not necessarily simple. It may comprise two or more different groups. Snake poison contains two toxophore groups, one agglutinating red blood cells, the other causing its general toxicity. Diphtheria toxin also has two toxophore groups, the one causing the acute symptoms and the other, the toxones with a long incubation, causing the later paralyses and cachexias.

The nature of immunity to these antigens is conceived as follows: The haptophore group is bound to the cell receptor because of a specific affinity.

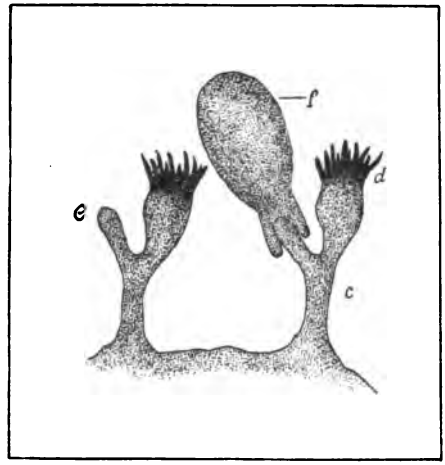


FIG. 55.—Illustrating receptors of the second order, Fig. 54, illustrating receptors of the first order. *c*, *d*, The cell receptor with a Zymophore group (*d*) and a haptophore group (*e*) capable of combining with disintegrated bacterial substances (*f*). The Zymophore group produces a ferment which acts upon (disintegrates) the bacterial cell or blood-corpuscle, as the case may be, seized upon by the haptophore group. (*Journal of the American Medical Association*, 1905, p. 1113.)

As a result this particular side chain or receptor is lost to the living cell and, following Weigert's law of supercompensation in regeneration, the cell replaces this loss by producing many more receptor groups than were previously present. As in the callus following a fracture there is an overproduction. In this way such a large number of receptors of one type are produced that they become excessive and the cell thrusts them off into the blood and into the fluids of the body. Here they constitute the specific antibodies and, because of their specific affinity, unite with the haptophore group of toxins and prevent their reaching the cell which they thus protect.

Therefore, in antitoxic immunity there are three stages: First, the chemical union of the haptophore group of antigen to the receptor group of

the protoplasm molecule; second, the overproduction and liberation of these receptors following this binding; and third, the union of these free receptors or antibodies with free toxin haptophore groups before these can reach the cell to injure them by the action of their toxophore groups. The antigens that are known with their respective antibodies as given by Hektoen are:

| *Antigens.* | *Products of Immunization.* |
|---|---|
| Toxins | Antitoxins |
| Ferments | Antiferments |
| Precipitinogens | Precipitins |
| Agglutinogens | Agglutinins |
| Opsonogens | Opsonins |
| Lysogens | Amboceptors or lysins |
| Antitoxins | Antiantitoxins |
| Agglutinins | Antiagglutinins |
| Complements | Anticomplements |
| Opsonins | Antiopsonins |
| Amboceptors | Antiamboceptors |
| Precipitins | Antiprecipitins |

These antibodies all result from the overproduction of simple receptors, but the protoplasm of cells may form still other cell receptors which are much more complicated and subserve the absorption of more complicated and complex albuminous molecules than those of toxins.

Bacterial clumping or agglutinating phenomena are extremely interesting as well as valuable in the diagnosis of disease. Upon this behavior of bacteria depends the Widal typhoid fever test. If the serum of an animal inoculated with the typhoid bacillus (antiserum) is added to a liquid culture or suspension of typhoid bacilli, the latter cease to move and after a time become aggregated into irregular clumps or masses. The same phenomenon is observed if instead of blood of a typhoid injected animal, the blood of a typhoid fever patient is employed. The reaction is quite specific, though not absolutely so. That is, similar agglutinating phenomena are produced by related bacilli, as the typhoid bacillus, the para-typhoid bacillus and the colon bacillus. Many other bacteria, beside the colon-typhoid group, are agglutinated by their respective antisera. In addition to diagnosing disease as in typhoid fever (the Widal test gives results even before there are marked disease symptoms), the agglutinating phenomena are useful in the identification of bacteria. The technic while not difficult, calls for many precautionary measures and requires considerable time and care to avoid erroneous conclusions.

In 1897 Kraus found that when the germ-free filtrates from broth cultures of bacteria were mixed with their respective antisera (serum from animals inoculated with the specific bacteria) the formation of a white precipitate occurred. The substance in the immunized serum which causes the forma-

tion of the precipitate has been termed precipitin. Similar reactions are observed with milk and egg albumen, when used with their specific immune sera. These reactions have been utilized to secure evidence in crimina cases. The serum of an animal which has been injected with human blood (humanized immune serum) produces a precipitate when mixed with human blood, even in high dilutions. Like agglutination, the reaction is, however, not wholly specific. For example, humanized animal serum will also produce a precipitate with the blood of higher apes. Dog immunized animal serum will produce a precipitate with wolf's blood, etc.

The chief immunizing agents are the bacterolysins, the antitoxins and the leucocytes (phagocytes) aided by the opsonins. The significance of

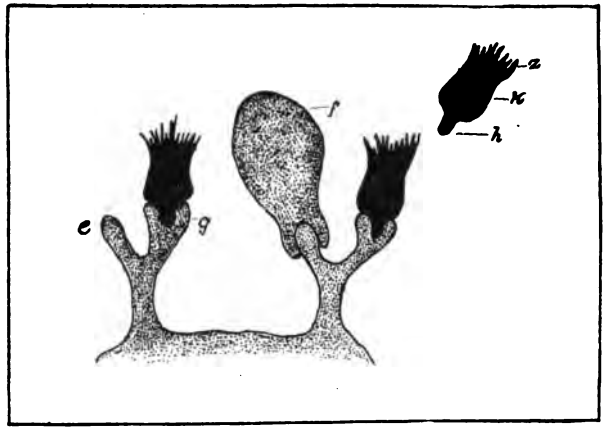


FIG. 56.—Illustrating receptors of the third order, or so-called amboceptors. This serves to explain the action of lysins (bacteriolysin, hemolysin, cell lysins, milk lysins, etc.). The cell receptor (amboceptor) has two haptophore groups, one (*e*) capable of uniting with a disintegrated substance as bacterial cell, blood-corpuscle, etc., (*f*) and the other (*g*) having the power to combine with a complement (*k*). *h* is the haptophore group of the complement (lysin) and *z* the zymotoxic group. Amboceptors, lysin receptors and receptors of the third order mean the same thing. (*Journal of the American Medical Association*, 1905, p. 1369.)

agglutinins and precipitins in the prevention of bacterial disease is not clear.

Recent observations on drug action tend to prove that some of these remedial agents apparently possess antitoxic and other immunizing properties. It is for example fairly well proven that phosphorus and *Echinacea angustifolia* have the power of increasing the opsonic index in certain bacterial invasions. Sulphide of carbon and silica appear to check and cure suppurative processes, perhaps due to similar activity. Nuclein which is usually derived from yeast, is reported to be decidedly bactericidal and to increase phagocytosis to a marked degree. According to Lloyd, Lobelia, when administered hypodermically, counteracts the toxin of the diphtheria bacillus,

being similar in its effects to the antidiphtheric serum (antitoxin of diphtheria). Belladonna is reported to be prophylactic as well as curative in scarlet fever. It is highly probable that as our knowledge of the therapeutic action of drugs develops, there will be a complete revolution in their use as remedial agents.

In conclusion, we give a summarizing table of the several immunizing agents above referred to and which will be more fully explained in the chapter following.

- Immunizing Agents
  - Active
    - Natural
      - Inherited or normal
        - Bacteriolysins.
        - Opsonins.
        - Phagocytes.
      - Augmented—Immunizing infectious diseases.
    - Artificial
      - Modified toxins and toxins.
        - Toxins.
        - Small-pox vaccination.
        - Rbies vaccination.
      - Bacterial
        - Bacterins or vaccines.
        - Bactericidal sera.
  - Passive
    - Antitoxins—Diphtheric, tetanic, etc.
    - Drugs—Nuclein, lobelia, phosphorus, etc.

## CHAPTER VIII.

## THE MANUFACTURE AND USE OF SERA AND VACCINES.

The most wonderful recent discoveries in the science of bacteriology pertain to the relationship of pathogenic germs and the serum of the blood of susceptible animals. As already stated blood serum has bactericidal properties (see lysins), but it is often not sufficiently active to destroy certain invading germs (pathogenic) and the disease manifestations, due to the toxins liberated by the germs, gradually develop. The bacterial toxins are of two kinds, those which escape from the bacterial cells and are soluble in the surrounding media, entering the system by absorption; and those which remain within the germ cell and are set free only on the breaking up of the bacterial cells. The former are the toxins proper or exotoxins, the latter are called endotoxins. As already explained the toxins cause the development within the serum of the blood of certain substances (antibodies), which neutralize or overcome the effects of the toxins and which are called antitoxins. Investigators hoped that experiments would prove that every pathogenic germ would cause the development of a corresponding antitoxin which might be used in the treatment of the disease. This hope has not been realized. Of the numerous experimentations with antitoxins only one has thus far proven entirely satisfactory, namely, the antitoxin of diphtheria. Several others have proven more or less useful, as will be explained later, but they are far from satisfactory.

The antitoxins act by neutralizing the bacterial toxins of the disease, and not by acting upon and killing the germs themselves. In this regard the antitoxins or antitoxic sera differ from the antibacterial or bactericidal sera, which act by preventing the development of the bacteria. This distinction and difference is not generally understood. The bactericidal sera have, however, thus far proven quite unsatisfactory in the treatment of disease. They are not standardized by units as are the antitoxins. The dose is by volume, from 10 to 50 c.c., and even more, usually given hypodermically. The sera are produced by injecting increasing amounts of germs (artificially cultured) into the animal, as the horse. As a rule the first injections consist of dead germs; finally, living germs of different virulency may be used. By this means a tolerance is established. The serum obtained from animals thus immunized is used in the treatment of disease, its action depending upon its bactericidal properties. There is a group of sera known as com-

posite, which give evidence of being a decided improvement over the simple sera. They are called composite because they have the peculiar qualities of two distinct forms of immunity—for example, diphtheria-immune horses may be used in the subsequent bacterial inoculation, which gives the resulting immune-serum a double content of a corresponding antibacterial body and of diphtheric antitoxin. This subject is as yet entirely in the experimental stage. It is also known that one kind or type of immunity has some influence not only upon other immunities, but also upon other diseases. The antitoxin of diphtheria, for example, appears to act as a cure or prophylactic against pathological conditions other than diphtheria.

We now come to a third class of substances used in the treatment of disease, namely, the bacterial vaccines, also designated bacterins and opsonogens (Ohlmacher). The term vaccine (from *Vacca*, a cow) is appropriately applicable to the small-pox remedy, but is entirely inapplicable to these newer agents. Either bacterin or opsonogen is a suitable name.

Bacterins are simply suspensions of dead pathogenic germs which are used in the treatment of disease. They produce their beneficent effects by acting upon the bacteria so that they may be taken up and digested by the white blood-corpuscles (phagocytes), as has already been explained. A homologous or autogenous bacterin is prepared from germs taken direct from the patient and is used in treating the same patient. A heterologous bacterin is one which is derived from a source other than the patient under treatment. A mixed bacterin is one in which the germs (of the same species) used are derived from several sources. The manufactured bacterins (heterologous) ready for use by the physician are called stock vaccines or stock bacterins.

The following is a tabulation of antitoxins, toxins, antibacterial sera and bacterins found upon the market and used by physicians and veterinarians.

### 1. For Human Use.

A. *Antitoxic Sera or Antitoxins.*

- Antidiphtheric serum.
  - Liquid or usual form.
  - Concentrated form.
  - Dry form (official in some pharmacopœias).
- Antitetanic serum.
  - Liquid or usual form.
  - Dry form.

B. *Antibacterial Sera or Bactericidal Sera.*

- Antistreptoccocic serum.
- Antipneumococcic serum.
- Antimeningitic serum.

Antityphoid serum.
Antidysenteric serum.
Antigonorrheal serum.
Antiplague serum (Yersin's serum).
Antianthrax serum.
Scarlet fever serum (Marpmann's serum).
Antituberculous serum (antituberculins).

C. *Bacterins or Opsonogens.* (*Vaccines*).
(Homologous or autogenous, heterogenous and mixed.)
Staphylococcus.
S. pyogenes albeus
S. pyogenes aureus. } used singly or mixed.
S. pyogenes citreus.
Streptococcus.
Gonococcus.
Typhoid.
Typhoid (Shafer's mixed bacterin).
Colon bacillus.
Neoformans bacillus.
Pyocyaneous bacillus.
Bubonic plague bacillus (Haffkine's plague vaccine).
Tuberculins.
Tuberculin, old (T. O.).
Tuberculin residuum (T. R.).
Tuberculin precipitate (T. P.).
Bacillus emulsion (B. E.).
Bacillus filtrate (B. F.).

D. *Toxins* (modified).
Small-pox vaccine.
On ivory points.
In glycerinated tubes.
Dry form.
Hydrophobia vaccine.
Erysipelas and Prodigiosus toxin. (Cancer and other malignant growths).
Antivenine. (Snake toxin.)
Cancer vaccin (Gilman's vaccine).

### 2. For Veterinary Use.

A. *Antitoxic Sera or Antitoxins.*
Antitetanic serum.
Influenza serum. (Intravenous use.)

B. *Antibacterial Sera or Bactericidal Sera.*

Antistreptococcic serum.
Canine distemper serum.
White scour serum.

C. *Bacterins.*

Anthrax.
Mallein.
Tuberculin.
Blackleg.
Blacklegine.
Blacklegules (pill form).
Blacklegoids (pill form).
Hog cholera.
Fowl cholera.
White scour.
Texas fever.

The above substances resemble each other in that they are organic and of complex chemical composition. They gradually deteriorate and finally become worthless, some sooner than others. Even the comparatively permanent kinds will not retain their full properties more than a few months, though they may still be sufficiently active therapeutically after eighteen months or even longer. They should be kept in a cool dry place, away from light. Turbidity in those preparations, which are clear when freshly prepared, indicates that decomposition changes have set in and that they are unfit for use. Many of the bacterins are normally turbid and nearly all of them have some slight color and odor.

Thus far only a few of the substances above tabulated have proven entirely satisfactory in the treatment of the particular disease or diseases for which they were intended. This is but to be expected since their use is very largely based upon theory. Theory and practice have ever failed to develop along exactly parallel lines. Science is however fortunate in being able to assert that in the antidiphtheric serum we have practically a specific for the cure of diphtheria, provided it is used in time and given in sufficiently large and sufficiently frequent doses. The antitetanic serum has given excellent results particularly as a preventive, as has also the antistreptococcic serum. Of the bacterins the Staphylococcus has given excellent results in the cure of actual pathologic conditions. Some of the others have proven less satisfactory and in many cases their great usefulness lies in their preventive rather than curative powers. The tuberculins, in particular, give promise of great usefulness in the eradication of the dread white plague.

We will explain very briefly the manufacture of a few of these substances only, as the methods are quite closely similar for like agents. The following

is a brief outline of the manufacture of the marvelous remedy for the treatment of the dread disease of childhood, namely diptheria.

### 3. Antidiphtheric Serum.

A. *Selecting and Testing the Horse.*—Ordinary, normal, non-pedigree horses are preferred, purchased under a guarantee of soundness. Even though purchased under such a guarantee the animal is kept under observation for a few weeks and tested for glanders by the mallein test. No animal is retained until it is proven that there is no latent or active disease present. The animal is well housed and well cared for during the entire time, under conditions as sanitary as it is possible to make them. All laboratories are also regularly visited by a U. S. Government inspector, who reports his findings to Washington.

B. *Preparing the Toxin of Diphtheria.*—Pure cultures of a breed or strand of the diptheria bacillus, possessed of a high potency, virulency or toxicity, are made in liter flasks containing beef bouillon. The original bacilli thus used are taken from some patient suffering with diptheria, and by means of isolation methods all foreign microbes are rejected or excluded. After the culture is several days old or when a maximum amount of the toxin has been formed and deposited in the bouillon, the bacilli are killed by adding 0.25 per cent. of trikresol. The bouillon with the dead bacilli is filtered. The clear filtered substance constitutes the toxin which is injected into the horse for the purpose of developing (in the horse) the antitoxin of diptheria. The virulency or potency of the toxin varies and is tested on guinea-pigs and compared with the U. S. Government standard. The desirable breed, race or strain of germs is perpetuated in the laboratory by daily transfers to new culture tubes. In this manner the bacilli are maintained for a long time, several years or longer. However, even with the greatest care the race finally deteriorates, weakens or undergoes a change in potency and it becomes necessary to secure a new stock culture.

C. *Developing the Antitoxin of Diptheria in the Horse.*—Twice weekly the horse is given (by hypodermic injection into the flank region) gradually increasing doses of the toxin of diphtheria. The rule is to give enough to produce a marked reaction. For a day or two the horse is sick with diphtheria, then recovers as the increased antitoxin in the blood (serum) of the animal neutralizes the toxin. This is continued for from four to six weeks when a maximum amount of antitoxin has presumably developed. The last dose of toxin is several hundred times greater than the first.

D. *Bleeding the Horse.*—A sterilized canula or trochar is inserted into the jugular vein, after the neck has been thoroughly washed with soap and water, shaved and rinsed with a 5 per cent. solution of carbolic acid. The

blood is drawn off into sterilized liter tubes, which are plugged with cotton. From nine to twelve liter of blood are taken from the horse at one time and the bleeding is repeated four or five times at intervals of about six months. The punctured wound is closed by keeping an artery forceps in position for a short time.

E. *Securing the Serum.*—The blood tubes are set aside until the clot has formed and settled to the bottom. The clear serum is siphoned off into a large flask, 0.25 per cent. of trikresol is added as a preservative and to kill

FIG. 57.—Bleeding the horse after a maximum amount of the antitoxin of diphtheria has been developed in the blood. The animals pay but little attention to the operation.

any germs that might be accidentally present, and then filtered through several thicknesses of filter paper, under pressure (suction). The perfectly clear, sterile and germ-free serum constitutes the antitoxin of diphtheria and is ready for use as soon as it is standardized and put into suitable containers.

F. *Standardizing the Antitoxin of Diphtheria.*—Since the antitoxic valence of horse serum as above described varies somewhat, it is necessary to determine the quantitative value in order that physicians may know what amounts to administer in the treatment of diphtheria. The standard unit of strength

now adopted by all civilized countries is the so-called Ehrlich unit, which is the amount of serum (antitoxin of the horse) which will just neutralize one hundred times a fatal dose of toxin when administered to a guinea-pig, weighing approximately 250 grams or one-half pound. The U. S. standard is prepared in the biological laboratories of the U. S. Marine Hospital Service

FIG. 58.—Sterilized liter tubes into which the blood drawn from the horse is placed. The top, covered with sterilized cloth, is connected with the canula in the jugular vein of the animal.

at Washington, and every manufacturer of diphtheric antitoxin in the United States is supplied with standard units from this laboratory. The method of procedure is approximately as follows: Eight containers (test-tubes) are set out in a row and numbered or marked serially. Into each tube is poured just one hundred fatal doses of toxin (fatal to a 250 gram guinea-pig, deter-

mined experimentally), and a graded amount of the serum to be standardized, so that the first tube has, in all probability, not enough antitoxin to neutralize the one hundred fatal doses of the toxin, and the eighth tube has, in all probability, a great excess of antitoxin. The contents of one tube is injected into a guinea-pig, thus requiring eight pigs. The animals are marked and kept under close observation. The first, second and perhaps third die, showing that not enough serum was added to neutralize the toxin. The fourth pig just recovers, showing that the amount of serum added to the fourth tube was sufficient to neutralize one hundred fatal doses of the toxin.

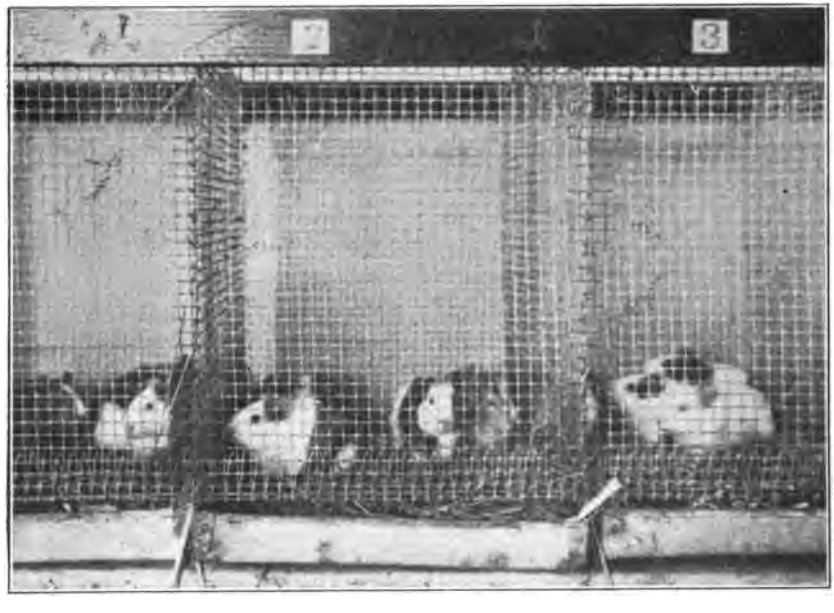


FIG. 59.—Guinea-pigs in wire cages. These lively little animals are used in testing the virulense of the diphtheria toxin which is injected into the horse and also for the purpose of standardizing the antitoxin. The reasons why these animals are preferred are wholly biological and physiological. They propagate rapidily, are easily kept, easily handled, and respond (biologically) to the tests applied.

This amount of serum (antitoxic) represents one unit. From this amount or unit the quantities to be put into the containers are determined. 500, 1000, 2500 and 5000 unit quantities are put up, for the convenience of physicians. 500 units constitute an immunizing dose, given to those who do not have diphtheria, but who have been exposed to the disease. The larger doses are curative. The rule is to give large doses, repeated as often as may be necessary.

### 4. Concentrated Diphtheric Antitoxin.

While the chemical nature of antitoxin is not known, it has been determined that it is united, in some way, with the globulins of the blood. The attempts to isolate antitoxin have resulted in the manufacture of a refined or concentrated antidiphtheric serum which is used quite extensively though it does not meet with the unqualified favor accorded the antidiphtheric serum. The process of manufacture is as follows:

FIG. 60.—Container with diphtheria antitoxin, supplied with hypodermic needle, piston, all ready for immediate use by the physician. The plunger is simply a homeopathic vial with rubber stopper. (*Cutter Laboratory.*)

A. The antidiphtheric serum is saturated with ammonium sulphate which precipitates the globulins (containing the antitoxin) in the form of a white mass. It is then filtered and the filtrate rejected.

B. The precipitate left on the filter is redissolved in water and this solution is again treated with ammonium sulphate as in (a). The object in redissolving in water is to wash the globulins.

C. The second precipitation product is treated with a saturated salt solution which dissolves the antitoxin globulins. The solution is then filtered.

D. To the filtered solution 2.5 per cent. of acetic acid is added which again precipitates the globulins on the filter paper where it is partially dried by means of filter paper and towels pressed upon the mass.

E. The partially dried material is placed in a dialyzing bag and suspended in a water current, for several days. This removed the salts by osmotic action and at the same time the globulins enter into solution within the bag.

F. A preservative is added to the liquid and which is then passed through a Berkefeld filter. Some physiologic salt solution is also added. This is the final product.

G. After being tested bacteriologically to make sure that it is not contaminated, it is standardized as described under diphtheric serum.

The above process removes the following non-active substances: serum albumins, lecithin, cholesterin, traces of bile salts and acids, blood salts and the non-antitoxic globulins. The dosage of the concentrated antitoxin is less than that of the non-concentrated serum and it keeps longer. For the manufacture of the concentrated diphtheria antitoxin the returned serum is generally employed, that is serum which has exceeded the time limit of use.

## 5. Antitetanic Serum.

This is prepared similarly to antidiphtheric serum. The tetanus bacilli are grown in bouillon, in the absence of oxygen, since tetanus germs are anaërobic. The growth is then killed, filtered out and the clear toxic, germ-free bouillon filtrate is utilized in the immunization of the horse. Small doses, usually mixed with some antitetanic serum, are administered at first and gradually increasing the amount as the horse can stand it until large quantities are given, even as much as 700 or 800 c.c. After some months the horse is bled in the same manner as for antidiphtheric serum, the serum is separated and bacteriologically tested in the same way.

The unit of tetanus antitoxin is that quantity of antitetanic serum which is necessary to completely neutralize 1000 fatal doses of tetanus toxin for a 250-gram guinea-pig.

Antitetanic serum has not been a marked success as a curative agent. Its greatest usefulness appears to be as a prophylactic, for which purpose it should be given early, as soon as the injury (cut, gunshot wound, abrasion) has occurred.

The following are the more important antibacterial sera. A fuller description of the processes of manufacture is omitted as that is a matter of

no special importance to the pharmacist. Furthermore, manufacturers do not, as a rule, disclose full details of manufacture.

### 6. Antipneumococcic Serum.

This serum is obtained from horses immunized against the Pneumococcus and is employed in the treatment of pneumonia and other infectious disease in which this germ is present. The dose is about 10 c.c. repeated several times a day, given hypodermically. The serum must be kept in a cool dark place. When a tube is opened the contents should be used within twenty-four hours, sealed temporarily with sealing wax, paraffin or sterile wadding. This serum has not proven very satisfactory, though it is safe and worthy a trial. (See pneumonia.)

### 7. Antimeningococcic Serum.

Antimeningococcic serum is obtained from horses which have been immunized with cultures of *Diplococcus meningitidis intracellularis*, beginning with dead cultures, then using living cultures and finally with autolysate. Its use is said to have met with considerable success in the treatment of cerebro-spinal meningitis, when injected into the spinal canal in doses of 10 c.c., repeated daily. The serum acts as an antitoxin, it increases phagocytosis and also acts as a bactericide. It should be used early in the course of the disease.

### 8. Yersin's Serum (Antiplague Serum).

Yersin's serum is made by injecting horses, first with dead plague bacillus cultures (*Bacillus pestis*) and finally with the living organisms. It has been used with varying success in plague epidemics. Large doses (30 to 50 c.c.) should be administered (hypodermically) early in the course of the disease. Its chief value is, however, prophylactic. The liquid form of the serum may also be used for intravenous injection. The dry serum is said to keep indefinitely and must be dissolved before using.

### 9. Bacterins.

The bacterins are still, so to speak, on trial. Some have given excellent results while others are wholly unsatisfactory. The preference appears to be for autogenous bacterins. The majority of physicians are, however, compelled to use the so-called stock bacterins, or the manufactured bacterins ready for use, for the reason that few physicians have the time or

the equipment to prepare the homologous or autogenous bacterins. The method of preparing a homologous bacterin may be outlined as follows:

a. A tube, flask or plate with the suitable culture medium (agar or gelatin) is inoculated with the germs taken from the patient and incubated, until a maximum development has taken place, about twenty-four hours.

b. The growth is separated from the culture medium by means of a sterile physiological salt solution and a platinum wire loop. The salt solution with the bacteria is transferred to a sterile test-tube which is then sealed in a flame.

c. When the tube is cool, it is shaken vigorously so as to emulsify the bacteria in the salt solution.

d. The tube is opened and about one drop is removed with which to make the blood-corpuscle count, to be explained later. The tube is again sealed in the flame.

e. The tube is now placed in a water bath (opsonic incubator of special construction for this work) at a temperature of 60° C. for a sufficient length of time to kill the germs; one hour is usually adequate. This constitutes the bacterin and is ready for use as soon as it is standardized. Usually some preservative is added when the tube is opened and before the bacterin is injected (0.2 per cent. lysol, 0.4 per cent. trikresol, etc.).

f. From the above it must be evident that no two preparations contain the same number of germs per c.c. and hence the physician cannot know how many dead microbes are injected at a dose. Therefore the necessity of standardizing the bacterin, which is done as follows:

g. Mix one part of freshly drawn blood with one part of the bacterin (taken from the tube in d.), add two or three parts of physiological salt solution, and spread evenly on a slide. Examine under the microscope and determine the number of microbes per c.c. in terms of the number of red blood-corpuscles per c.c. This is done by making numerous (10 to 20) counts of red blood-corpuscles and microbes. Knowing that there are 5,000,000,000 red blood-corpuscles per c.c., it is then a simple matter to compute the number of microbes per c.c. in the bacterin under consideration. The count thus determined divided by the number of bacteria desired for one dose, indicates the number of times the bacterin is to be diluted. This is very clearly illustrated in a chart prepared by Houghton, shown in Fig. 61.

The number of bacteria administered per dose depends upon the therapeutic effects to be produced, the kind of bacterin used, the nature of the disease and the condition of the patient. The rule is to start with small doses, gradually increasing them in such a manner as to secure a maximum of positive opsonic phases with a minimum of negative opsonic phases. In round numbers the dosage ranges from 5,000,000 to 50,000,000 bacilli, represented by varying quantities of the bacterins.

| Red Cells | Bacteria |
|---|---|
| 16 | 12 |
| 14 | 8 |
| 9 | 5 |
| 17 | 15 |
| 16 | 10 |
| 20 | 9 |
| 11 | 10 |
| 19 | 13 |
| 15 | 9 |
| 6 | 5 |
| 17 | 14 |
| 8 | 6 |
| 18 | 11 |
| 18 | 17 |
| 22 | 10 |
| 18 | 8 |
| 14 | 16 |
| 14 | 10 |
| 18 | 16 |
| 18 | 20 |
| 308 | 224 |

308 : 224 :: 5000 : X = 3636

$\frac{3636}{400} = 9$

FIG. 61.—Counting the bacteria in standardizing bacterins. This chart shows the count of bacteria and of red blood cells in twenty successive fields of the microscope. The number of red cells counted (308) is to the number of bacteria counted (224) as the number of red cells per cubic centimeter in normal blood (5,000,000,000) is to the number of bacteria per c.c. in the suspension (3,636,000). This count (3,636,000) divided by the count desired in the final dilution (400,000,000) gives the number of times (9) this suspension must be diluted to bring it to the desired dilution. (*Parke, Davis & Co.*).

## 10. Tuberculins.

The tuberculins are of special interest as they give great promise in the successful treatment of tuberculosis. The different kinds have their special use. Their manufacture is briefly outlined as follows:

A. *Tuberculin Old* (T. O.).—This is the original Koch tuberculin or Koch lymph and is a concentrated bouillon culture of the tubercle bacillus, which has been filtered to remove the germs. It is a toxin solution and not a bacterin proper.

B. *Tuberculin Residuum* (T. R.).—This is prepared by grinding the dried tubercle bacilli, extracting with water, centrifugalizing, discarding the supernatant liquid, regrinding the sediment, which is first allowed to dry, and mixing with glycerin and water. It is thus a suspension of pulverized tubercle bacilli in an aqueous solution of glycerin. The grinding process is tedious and requires much time. The tuberculin is standardized so that 1 c.c. will represent 10 mg. of the dry culture.

The supernatant liquid, after centrifugalizing, is sometimes drawn off, instead of rejecting, and constitutes the upper tuberculin (T. O.) (Obere Tuberculin). These two tuberculins (the T. R. and the T. O.) differ in therapeutic value and in physical properties.

C. *Bacillus Emulsion* (B. E.).—This consists of pulverized tubercle bacilli suspended in 50 per cent. glycerin and is standardized to contain 5 mg. of solid matter per c.c. It differs from T. R. in that the supernatant liquid (T. O.) is not drawn off.

D. *Tuberculin Precipitate* (T. R.).—This is obtained from old tuberculin by precipitation with alcohol, drying and pulverizing the precipitate. It is used in making the Calmette eye-test. (See tuberculosis.)

E. *Bouillon Filtrate* (Tuberculin Filtrate B. F.—Denys Tuberculin).—The tubercle bacillus cultures are passed through a Berkefeld filter to remove all germs. The filtrate is preserved with trikresol.

## 11. Small-pox Vaccine.

Small-pox vaccine is not a true toxin nor yet a true bacterin. Its value in the eradication of small-pox has world-wide recognition. The following is the manner in which small-pox vaccine is prepared.

A. *Selecting the Animal.*—A young heifer (five to ten months old) is selected, tested for tuberculosis by means of tuberculin. The animal is observed for a time to make sure of general condition of health; is well fed and well cared for, under conditions as sanitary as it is possible to keep them.

B. *Inoculating the Animal.*—The heifer is strapped securely to a framework, back down, the udder region is cleansed, shaven and cross marked (scarified) with a sharp scalpel. The cuts are just deep enough to cause the

escape of serum, not actual bleeding. This scarified surface is then inoculated with glycerinated small-pox virus taken from a patient. When the inoculated material has had time to be absorbed the animal is righted again and cared for under as aseptic conditions as possible. In time (six to seven days) pustules form over the entire inoculated area. The virulent virus from man conveys the disease to the animal, but in its passage through the animal it becomes modified, losing in virulency, yet capable of producing immunity as the result of a mild intoxication (vaccinia).

C. *Removing the Scab.*—The animal is again fastened to the frame.

FIG. 62.—Showing the heifer strapped to the frame, preparatory to removing the vaccinia scab from the area which was scarified and inoculated with the small-pox virus. The scab patches show dark.

The inoculated surface is washed and dried. The thick scab which has formed over the inoculated area is then removed and triturated with 50 per cent. glycerin. This constitutes the small-pox vaccine.

D. *Aging or Ripening the Vaccine.*—The fresh or raw vaccine is not used as it contains various living microbes. It is acted upon by the glycerin added, for five or six weeks. The virus is tested bacteriologically during this period, and as soon as no more colonies appear it is ready for use.

E. *Preparing for the Market.*—The vaccine is now put into small glass

tubes and marketed as glycerinated tube virus. The vaccine should be kept, in a cool, dark, dry place. It deteriorates gradually and the time limit of usefulness is stamped on each package.

The old time ivory tips are still on the market and are preferred by many physicians. A dry bulk form of the virus is also marketed. The manner of the use and the action of the virus are universally known. As now prepared the remedy is absolutely safe. No ill effects ever follow its use. Of the millions of persons inoculated within recent years, there probably has not been a single instance of bad effects which could be traced primarily to the vaccine virus itself. A small-pox vaccination is not nearly as likely to produce ill effects as the customary hand shake. In fact the latter operation does occasionally spread an infection.

## 12. Hydrophobia or Rabies Vaccine.

Pasteur's hydrophobia virus is obtained from the spinal cord of rabbits, inoculated with the virus from a dog suffering with rabies. The inoculation is made into the dura mater of the spinal cord. The rabbit dies in about two weeks. A second rabbit is inoculated from the first, which dies even sooner, showing that the toxin gained in virulency in its passage through the first animal. This is repeated until finally the animal dies in six or seven days after inoculation. Beyond this the virulency of the poison cannot be increased and this constitutes the *virus fixe* (fixed or unchanged virus) of Pasteur.

The spinal cord of the rabbit dead of *virus fixe* is dried in a glass cylinder with potassium hydrate. The cylinder is placed in a cool dry place and each day small bits of the cord are cut off and placed in a vial of glycerin. At the end of fourteen days the virus is no longer capable of producing hydrophobia in rabbits, but the animal inoculated with it can withstand the thirteen days virus (which was preserved in the glycerin) and so on down the scale, until finally the rabbit can withstand the *virus fixe* without experiencing serious effects.

In man it is customary to begin the treatment for rabies (or suspected rabies) with the nine day cord (hypodermic injections of the cord emulsions) and to give each succeeding day a virus one day stronger, until finally the *virus fixe* is injected without producing untoward symptoms. The individual thus treated is now able to withstand the much weaker virus from a dog or other animal suffering from rabies. As the result of this mode of treatment the mortality rate from rabies is now less than 1 per cent. (Ravenel). Those bitten by dogs (or wolves, skunks, cats) suffering from rabies or suspected of suffering from rabies, should cleanse, cauterize and disinfect the wound at once, and then immediately proceed to a Pasteur Institute and

submit themselves for treatment. The earlier, after infection, the treatment is begun the more likely will the results be satisfactory. The vaccine is, however, now so prepared as to make home treatment possible. The graded doses of the virus put up in sterilized ampuls are ready for immediate use by the family physician.

### 13. Streptococcus Mixed Vaccine.

A mixed toxin of erysipelas (*Streptococcus pyogenes* and *Bacillus prodigiosus*) is used in the treatment of cancer and other malignant tumors, particularly the sarcomas. It appears to have a local as well as systemic effect. It is prepared as follows: Virulent cultures of the *Streptococcus pyogenes* are grown in the incubator for three weeks, then inoculated with the *Bacillus prodigiosus* and allowed to grow ten days longer at the room temperature. The mixed cultures are then bottled, sterilized by heating for an hour at 60° C., and are ready for use. Being unfiltered, the preparation is decidedly turbid in appearance. This preparation is administered hypodermically, the injection being made in the neighborhood of the tumor or into the tumor itself.

### 14. Cancer Vaccine.

Recently Dr. Gilman of Johns Hopkins University has experimented with a cancer vaccine or cancer emulsion which it is believed will cure cancer. It is simply a preparation made from the cancerous tissue of the patient, macerated in a physiologic salt solution and preserved in some antiseptic. This is suitably diluted and given in 10 c.c. doses injected directly into the circulation. A reaction (rise in temperature to 102° to 104° F.), develops in three days, suppuration ceases and the tissues begin to heal. The dose is repeated three times at intervals of two weeks which is supposed to be sufficient to effect a cure. Although not a bacterin as far as can be ascertained, its action is evidently similar to that of an autogenous bacterin. As yet it is too early in the history of the use of this remedy to predict the final results.

## CHAPTER IX.

## YEASTS AND MOULDS.

The organisms commonly designated as yeasts and moulds, though not belonging to the bacteria (Schizomycetes), are of the greatest importance in human economy and play a most active part in life. Some of them are most

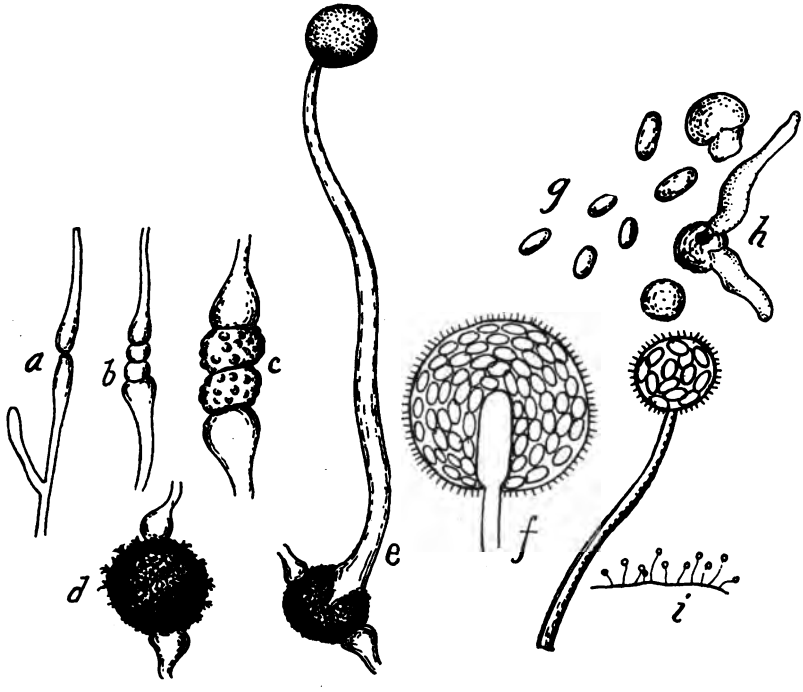


FIG. 63.—Development of *Mucor mucedo*. *a*, *b*, *c*, *d*, stages in the formation of the zygospore; *d*, mature zygospore; *e*, *f*, endospore formation; *g*, endospores; *h*, germinating spore, this develops and finally gives rise to new zygospores; *i*, mucor slightly magnified. This mould is found on stale bread, damp leather, gloves, etc.

beneficent while others are very injurious to health. The yeast organisms (Saccharomyces) cause the alcoholic fermentations in saccharine solutions. Many of the mould group cause skin and other diseases. They all belong to the plant division fungi. The more important species may be grouped under three orders, as follows:

I. Phycomycetes. (Zygomycetes.) Zygospore formation.

1. *Mucor corymbifer.*
2. *Mucor mucedo.*

} Both are found in tissue infections.

3. Other species of Mucor are reported as causing pathologic conditions in man and in lower animals. Some are the cause of fatal infectious diseases in such household pests as the common fly. Others attack fruits, as pears, figs in particular, leather goods as gloves, etc.

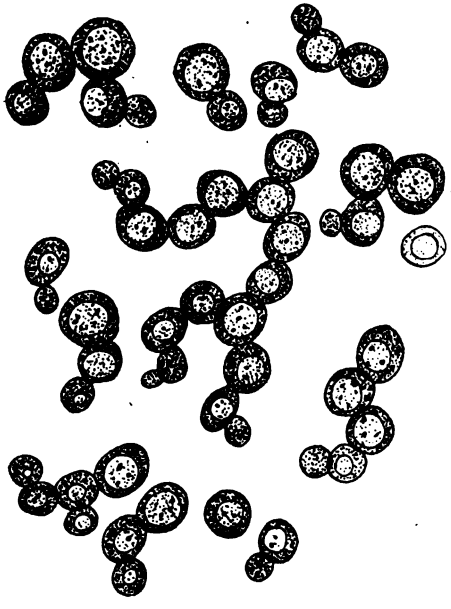

FIG. 64.—*Saccharomyces cerevisiæ.* The form or variety known as brewers' top yeast. (*Oberhef.*)

II. Ascomycetes. Spores formed in asci (sacs).

1. Saccharomycetes—yeasts proper.
   a. *Saccharomyces cerevisiæ.* This name is applied to many species or varieties of yeasts concerned in fermentation processes, as in beer, wine and saké making.
   b. *Saccharomyces anginæ.* Pathogenic.
   c. *Saccharomyces ellipsoides.* Common in fermenting fruits, jams, jellies, fruit juices, etc. Other species are active in various vegetable food fermentations.
   d. *Saccharomyces blanchardi.* Pathogenic.
   e. *Endomyces albicans.* Pathogenic, causes thrush.

f. *Cryptococcus gilchristi.* Pathogenic; general infections.

g *Cryptococcus hominis.* Pathogenic.

2 Gymnoascomycetes.

a. *Trichophyton tonsurans.* Pathogenic, causes scalp disease (ringworm), also attacks other external tissues.

b. *Trichophyton sabourandi.* Pathogenic. Attacks scalp and beard (ringworm).

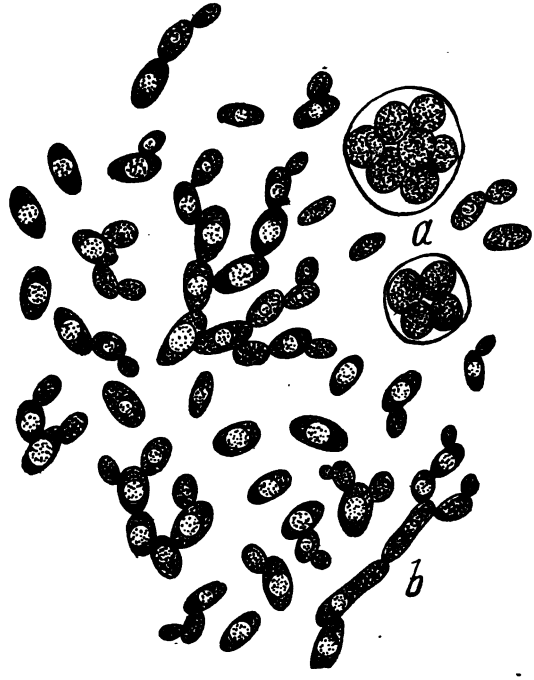


FIG. 65.—*Saccharomyces cerevisiæ.* The form or variety known as brewers' bottom yeast. (*Unterhefe*). *a*, Spore formation; *b*, elongated cells, which develop under certain conditions of moisture, food supply, etc.

c. *Trichophyton violaceum.* Pathogenic. Like (b). Violet color.

d. *Trichophyton mentagrophytes.* Pathogenic. Causes beard and body ringworm.

e. *Trichophyton cruris.* Pathogenic.

f. *Microsporum audouini.* Pathogenic.

g. *Achorion schœnleini.* Pathogenic. Is the cause of that very common scalp disease of children known as favus.

3. Carpoascomycetes.

a. *Penicillium crustaceum.* This is a blue-green mould which is

believed to be pathogenic in chronic catarrhal conditions of the Eustachian tubes and of the stomach.

b. *Penicillium glaucum.* This is the omnipresent blue-green mould so common in the household, infesting all exposed moist organic substances. Supposed to be non-pathogenic, although some credit it with being the cause of pellagra.

c. *Aspergillus fumigatus.* Said to be the cause of pellagra.

d. *Aspergillus concentricus.* Causes ringworm. Common in the

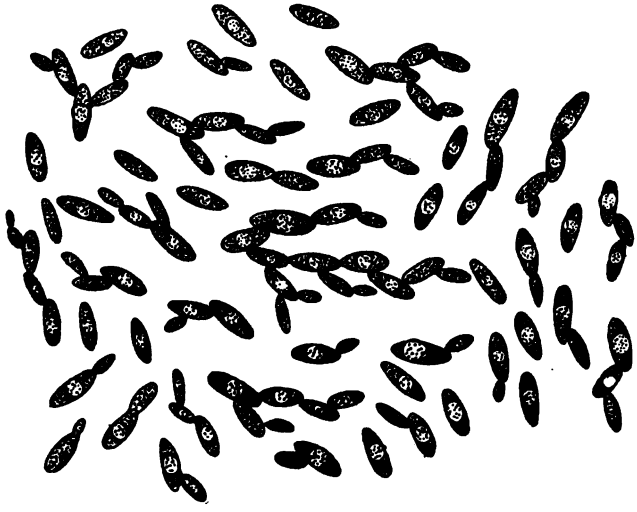

FIG. 66.—*Saccharomyces ellipsoides.* Very common in fruit products as jams, jellies, etc. Living yeast cells show budding of cells and vacuoles. Dead yeast cells usually occur singly, the vacuoles are wanting and the cell walls are more distinct, generally due to the absorption of coloring substances in the medium in which they occur.

Malay peninsula, China and in the Philippines. Limited to tropical countries.

d. *Aspergillus flavus.* Pathogenic. Found in chronic discharges from ear.

e. *Aspergillus repens.* Much as (d).

f. *Aspergillus pictor.* Pathogenic. Occurs in Central America, where it causes a mange disease.

g. *Aspergillus oryzæ.* Nonpathogenic. Cultures of this fungus are used in the manufacture of saké (Chinese and Japanese rice wine). The fungus growing and feeding upon the steamed rice grains converts the starch into saccharine substances which are then acted upon by the yeast ferment.

III. Hyphomycetes. Systematic position of the pathogenic members not well defined. Life history not yet fully worked out.

1. *Discomyces bovis.* (Actinomyces). The so-called ray fungus which causes the condition in cattle known as actinomycosis, a disease which can be transmitted to man.

2. *Discomyces maduræ.* (Mycetoma). Causes the cattle disease known as madura foot, which can be transmitted to man. Essentially a tropical disease. Two varieties (black and white) of the disease are reported.

3. *Malassezia furfur.* This is the fungus which causes a skin disease

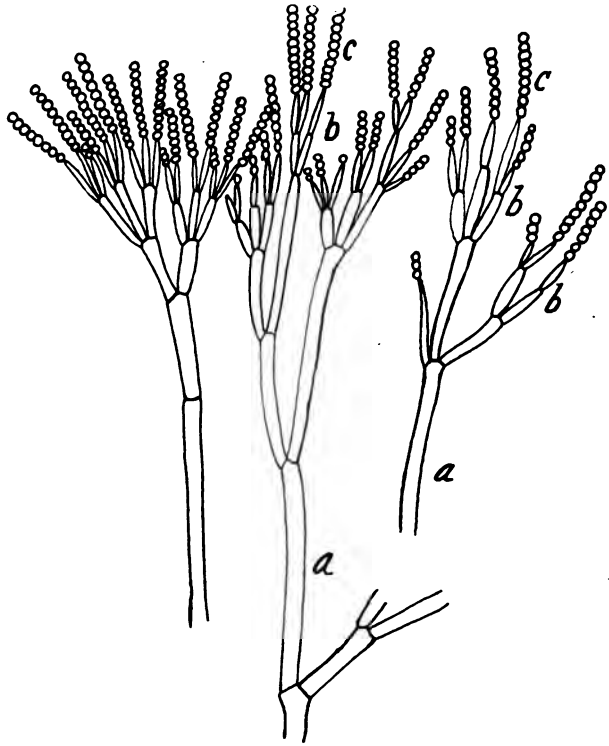


FIG. 67.—Three terminal hyphæ showing the characteristic spore formation of *Penicillium glaucum.* This fungus is a true saprophyte and is never found on living fruits or vegetables. Mouldy food substances are quite universally rejected as being unfit for human consumption.

(*Tinea versicolor*) which is quite common in tropical as well as in temperate climates.

4. *Microsporoides minutissimus.* Causes a skin disease known as Erythrasma or Dhobie's itch. Found in the tropics.

5. *Trichosporum giganteum.* Causes a disease of the hair. The spores of the fungus are arranged about the hair in a peculiar mosaic.

Moulds differ from bacteria in that they thrive best in acid media and in that they are not so readily killed by means of the usual chemical disinfectants. Heat (dry as well as moist) kills the hyphal structure quite readily, but the spores are quite resisting, though less so than the spores of bacteria. They can be cultured on potato, on bread, or on other organic food materials (kept moist in a moist chamber). The following medium is very satisfactory.

| | |
|---|---|
| Maltase | 4 gm. |
| Peptone | 1 gm. |
| Agar | 1.5 gm. |
| Water | 100 c.c. |

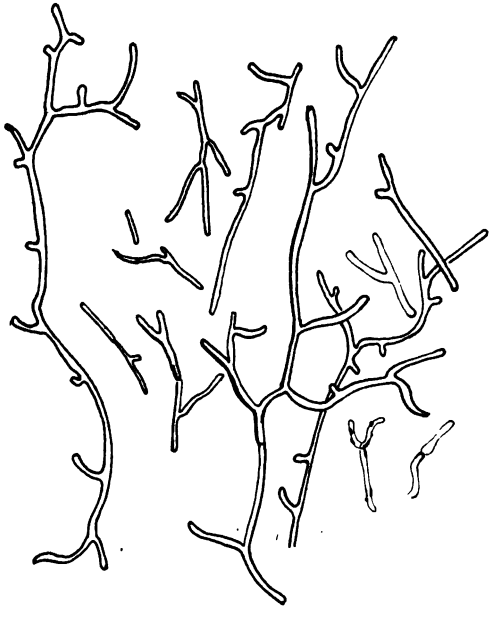

FIG. 68.—*Actinomyces bovis.* Showing the hyphal structure of this pathogenic fungus. There are numerous fungi of the mould group that cause local pathologic conditions of the skin and mucous membranes.

Mix, dissolve, filter, titrate to reaction +2 and sterilize in the usual way. Culturing is usually done in Erlenmeyer flasks (250 or 500 c.c.) with a thin layer of the medium in the bottom. Before placing the mould material in the flask (by means of a platinum loop) allow it to macerate in 60 per cent. alcohol for two hours which will kill the bacteria present without destroying the life of the mould. The acid reaction of the medium (+2) will, however, usually prevent bacterial growth.

Yeast organisms may be studied very conveniently in the hanging drop. The development of mould may be observed between two sterile slides. Since these organisms are much larger than bacteria there is little difficulty in examining them under the low power of the microscope. Mount in water or in a weak solution (10 per cent.) of caustic potash or soda. In looking for yeasts and moulds in liquids, centrifugalizing may be desirable. Staining methods will rarely be necessary.

While it is true that not all moulds are pathogenic, yet it must be remembered that many are decidedly so, besides most of them are very objectionable on account of the disagreeable mouldy odor and taste, if for no other reason. Mouldy food substances are not fit for consumption and moulds should not occur in any of the pharmaceuticals, syrups, soda fountain preparations and fruit juices. Most of the yeasts are non-pathogenic. The common yeast has even been used as an intestinal disinfectant in typhoid fever, yet no preparations in the drug store should be allowed to undergo yeast fermentation for the reason that the process changes the quality and flavor of the substances thus attacked. Fruit pulp, fruit juices and syrups of all kinds are peculiarly liable to the attacks of the yeast organisms and every precaution should be taken to guard against such infection. This is not a simple matter because the yeast cells and the yeast spores are found everywhere and develop very readily in all saccharine, slightly acid substances. Moist heat sterilization or pasteurization are the most effectual means for preventing yeast fermentations.

The yeast cakes used by the housewife in making bread consist simply of pure cultures of Saccharomyces. The cakes must be kept dry and in the cold (ice chest) to prevent ready decomposition. Even under the most favorable conditions they soon become worthless. As soon as the cake is mixed with the bread dough with adequate warmth, the yeast cells begin to feed upon the various available food substances present and multiply rapidly (by budding), resulting in the formatin of alcohol and liberation of $CO_2$ gas, which latter in an attempt to escape, causes the so-called rising of the bread. If the dough is not thoroughly mixed, the gas liberation is uneven and the bread will be unsatisfactory, because there will be large cavities in some parts of the loaf and in other parts the loaf will be solid. Bread must be baked quickly, after the rising has reached the proper degree, otherwise the loaf will be flat and doughy. The housewife in the country simply prepares sour dough cakes which take the place of the manufactured yeast cakes used in the city. In biscuit making the desired $CO_2$ gas liberation is brought about by the use of baking soda and sour milk or by means of baking powder alone.

The alcoholic fermentation in the manufacture of beer is caused by the several varieties and forms of *Saccharomyces cerevisiæ* (*Torula cerevisiæ*).

In beer making, the barley grain is first acted upon by the starch enzyme (diastase) which converts the starch into maltose (malt) and the maltose is in turn converted into alcohol by the Saccharomyces. If the fermentation product (as grape wine, apple cider, beer, porter, etc.) is exposed to the air for a time, the *Mycoderma aceti* enters and at once begins to convert the alcohol into acetic acid and we finally have vinegar. "Hard cider" is simply apple wine in which the acetic acid fermentation has progressed to an advanced stage.

In the manufacture of the Japanese and Chinese rice wine (saké) the maltose fermentation of the starch (in the rice grain) is brought about by the *Aspergillus oryzæ* as already stated. The process of beer and saké manufacture may be compared as follows:

| BEER. | SAKÉ. |
|---|---|
| **1. Material Used.** | |
| Carefully selected barley is cleaned in running water, then macerated in water to induce germination. Rice, wheat and other cereals may be added. Hops are used. | A good quality of rice is thoroughly washed in cold water, then softened by a steaming process. No hops used. |
| **2. Diastase Fermentation. Malting.** | |
| During the germinating process a ferment or enzyme (diastase) is liberated which converts the starch into saccharine compounds. The ferment is unorganized (non-living) and is soluble in water. The germinating and fermenting grain constitutes the beer wort. | The steamed rice is spread on mats and inoculated with the spores and hyphæ of *Aspergillus oryzæ*. This fungus liberates an enzyme (diastase) which converts the starch into saccharine substances. The enzyme produced by the fungus is soluble in water. Fermentation takes place in a warm room. |
| **3. Alcoholic Fermentation.** | |
| The beer wort (Bierwürze) is now ready to be acted upon by the yeast organisms (*Saccharomyces cerevisiæ*) which enter from the air or which may be added in pure culture. The yeast organisms convert the saccharine substances into alcohol and carbonic acid gas ($CO_2$).<br>The diastase and the yeast ferments are both active during this process. | The saké wort (*moto*) is prepared by mixing the steamed rice and fungus (*A. oryzæ*) in vats. Yeast cells (*Saccharomyces* of saké) enter from the air and cause alcoholic fermentation, converting the saccharine substances into alcohol and carbonic acid gas ($CO_2$).<br>The diastase ferment (produced by *A. oryzæ*) and the alcoholic ferment (Saccharomyces) are active during the entire process. |

### 4. Expressing, Cooling, Clarifying and Pasteurizing.

These processes are very closely similar in beer and saké brewing. The differences, if any, are slight and pertain to modifications of methods employed by different manufacturers. Preservatives, as salicylic acid, may be added. Both beverages may be reinforced with alcohol. This is not generally done with saké as the brewers declare that the addition of foreign alcohol destroys the characteristic flavor or bouquet.

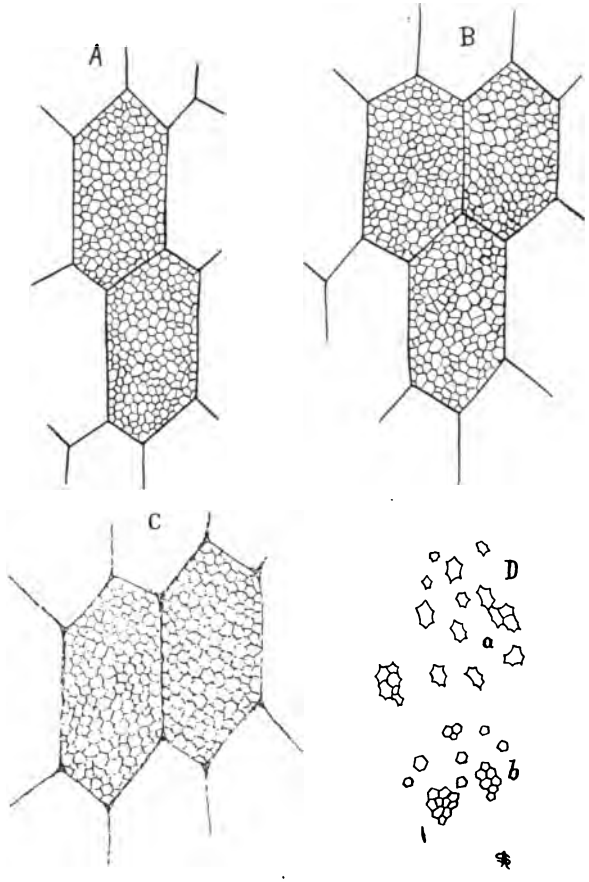


FIG. 69.—Saké making. *A*, *B*, Rice cells entirely filled with starch granules; *C*, rice cells after steaming, the starch granules are broken up; *D*, rice starch granules *a*, dextrinized, *b*, normal.

### 5. Kinds or Brands.

Many different brands varying in color, taste, alcoholic percentage, ash percentage, etc. The alcoholic percentage ranges from 1.5 to 6. The ash percentage is about 8.

Different brands varying in quality. The alcoholic percentage ranges from 14 to 18. There is a sweet variety (*Mirin*) and a white variety (*Shiro*). Ash percentage about 3, frequently less.

### 6. Use and Properties.

A beverage, usually taken in comparatively large doses, producing a mild form of intoxication.

Usually taken in small amounts, producing a speedy, though transient, form of intoxication. Taken as a wine. In Japan saké is usually heated before drinking.

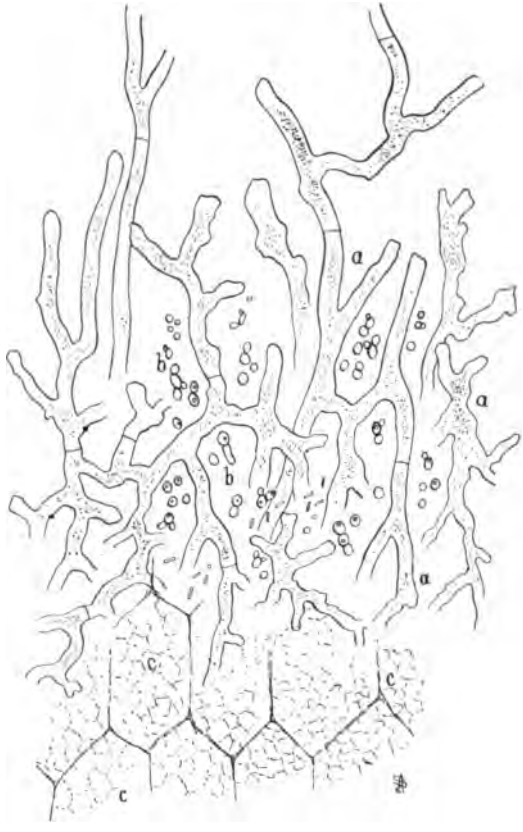


FIG. 70.—Saké making. Steamed rice cells (*c*) attacked by the hyphæ (*a*) of *Aspergillus oryzæ* which feed upon the dextrinized rice starch, converting it into sacchrine substances. Yeast cells and bacilli are usually associated with the hyphal fungus, feeding upon the saccharine substances formed.

There are numerous varieties of *Saccharomyces* concerned in beer brewing. There are several kinds of upper or top yeasts (*Kahmhefe Oberhefe*) and several kinds of bottom or lower yeasts (*Unterhefe*), each kind possessing supposedly special properties. Just what part the more or less incidentally associated organisms (as bacteria, moulds, and foreign yeasts) may play in the fermentation processes is not clearly understood. It is known that some

of these extraneous organisms may develop to such an extent as to modify the quality of the product completely. Such fermentation diseases are a source of much annoyance to manufacturers, often resulting in great financial loss, but this has also been the great stimulus in compelling the use of pure cultures and in perfecting those methods which are known to improve

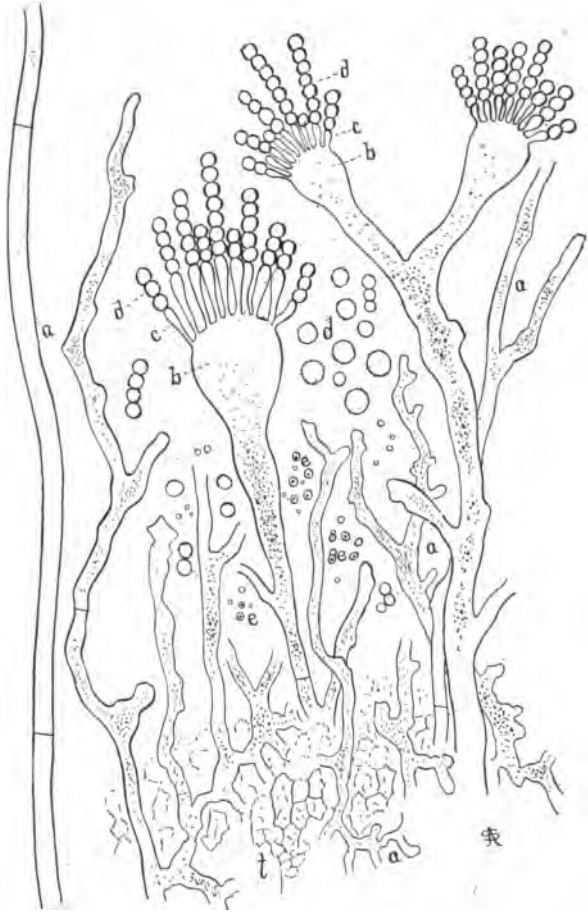


FIG. 71.—Saké making. *Aspergillus oryzæ*, showing vegetative hyphæ (*a*), and spore-forming hyphæ (*b*, *c*, *d*).

the keeping qualities of the articles, whether foods or drink. Saké in particular, does not keep well, even with the greatest care in manufacture and with the use of preservatives. Certain brands of beer, wine, saké, smoking tobacco, cigars, tea, etc., are known to lose their characteristic flavors within short periods, due to the invasion of some "disease" producing organism.

In many instances manufacturers have been blamed for inferiority in the quality of fermented products when in reality said articles left the establishment in perfect condition as far as quality is concerned, but were subsequently (in shipment, in storage, etc.) attacked by some objectionable organism, resulting in a complete change of flavor or bouquet.

FIG. 72.—Saké making. *A*, Dead or dying yeast cells; *B*, active yeast cells which convert the saccharine substances formed by the aspergillus into alcohol. *C*, *D*, yeast cells and hyphæ of aspergillus from the fermenting vats.

The Japanese soya sauce (fermented soya beans, *Glycine hispidus*) and miso, a soup stock of wheat and soya beans, is prepared through the action of *Aspergillus oryzæ* and *A. wentii*. The Javanese arrak is made from rice which is first acted upon by a fungus (*Ragi*) in many respects similar to *A. oryzæ*, and subsequently, the alcoholic fermentation is carried on by the Saccharomyces, thus the method of arrak manufacture is closely similar to

that of saké. More generally, however, arrak is made from fermented molasses. There are many other species of mould, including the very common *Penicillium glaucum*, which have the power of converting starch into saccharine compounds in the presence of moisture, but thus far these are not used industrially. An alcoholic drink of the East Indies is prepared from a starchy root as follows: A number of people, usually girls, sit about a large

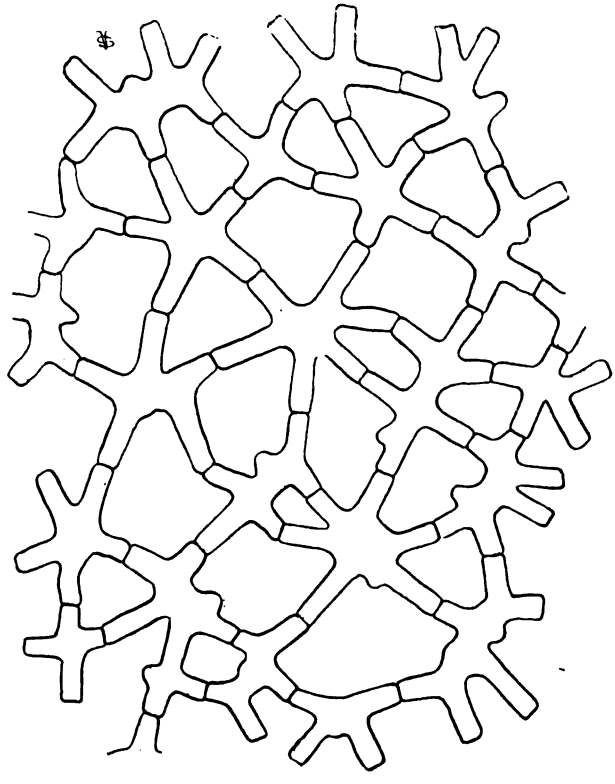

FIG. 73.—Showing the characteristic stellate cells of the pith of same reed used as filtering material in clarifying saké. Bundles of the pith are placed in the bottom of a perforated cask, forming a layer a foot or more in depth; through this the saké percolates. The impurities are caught in the intercellular spaces of the pith.

vessel masticating the roots which are then expectorated into the vessel. The ferment ptyalin of the saliva converts the starch into saccharine substances which is then acted upon by the Saccharomyces, resulting in an alcoholic drink which is said to have a very peculiar flavor. Pressed yeast cakes for bread making are prepared as follows:

The filtered saccharine yeast mash in vats, is inoculated with pure cultures of *Saccharomyces cerevisiæ*. Active fermentation takes place in the

presence of pure air which is supplied through pipes leading into the vat. The white scum or foam which forms is poured on fine sieves, washed with sterile water, and then centrifugalized to remove most of the water. This partially dry material is then pressed into cakes, thoroughly dried at a low temperature, and wrapped in lead foil to exclude air. Starch is sometimes added as a dryer, but this is no longer necessary because of the improved methods of manufacture. Good yeast should be of a yellowish color, easily powdered and should have a pleasant "yeasty" odor.

The so-called Chinese yeast, concerned in various fermentation processes is a mixture of Mucor species, yeasts and bacteria. The following species of mucor are prevalent—*M. racemosus*, *M. alternans*, *M. spinosus*, *M. circinelloides* and *M. boidinii*. These have the power of converting starch into saccharine compounds, which are then acted upon by the Saccharomyces. Various alcoholic ferments have been employed in China and Japan since time immemorial.

Nuclein is prepared from yeast and other vegetable cells and is very much used in the treatment of certain diseases due to pathogenic bacteria. It is said to have strong bacteriolytic properties and to increase phagocytosis.

## CHAPTER X.

## PROTOZOA IN DISEASE.

Certain low forms of animal life are causative of such diseases as malaria and sleeping sickness. These organisms resemble each other in that they are minute, of simple structure (single celled) and in that they show active motion due to the presence of flagellæ or cilia or due to cell undulations. They are found in stagnant water containing decaying vegetable and animal matter and in decaying organic matter. Most of them are non-pathogenic and all are quite readily killed by means of heat and the common chemical disinfectants. They do not occur in pure, fresh well, spring, or hydrant water. If present it is a sure sign of vegetable contamination. All liquids intended for internal use, showing the presence of amœba, infusoria and other protozoa should be tested bacteriologically for the presence of colon bacilli, the typhoid bacillus and other possible pathogenic organisms, animal as well as vegetable.

The following are the more important species of protozoa and the principal activities in which they are concerned:

I. RHIZOPODA.—These move by throwing out slender protoplasmic projections. Silicious coverings may be present.

1. *Entamœba coli.*—Inhabits the large intestine. Probably harmless. May be confused with phagocytes.

2. *Entamœba histolytica.*—Causes entero-colitis and dysenteric ulcerations. It is also found in abscesses of the liver. Occurs in tropical countries, less common in temperate zones.

3. *Entamœba buccalis.*—Found in dental caries. Probably not pathogenic.

4. *Entamœba undulans.*—Occurs in the intestinal tract.

5. *Leydenia gemmipara.*—Identity doubtful. Supposed to have a causal relationship to carcinomatosis (cancer).

II. FLAGELLATA.—Motion due to flagellæ. Some possess an undulatory motion. Have been classed as bacteria (Spirillæ).

1. *Spirochæta recurrentis* (*Spirillum obermeieri*).—This is the organism which causes relapsing fever. The disease is so designated because after apparent complete recovery, one or more relapses invariably follow. It is not a very fatal disease (4 per cent. of deaths) and is, so far, rare in the United States. It is and has been very prevalent in parts of Europe. The disease can be transmitted, by inoculation, to man,

monkeys, mice and rats. An immunity treatment has been attempted with some success. Most authorities class the organism as a fungus (Spirillum).

2. *Spirochæta duttoni.*—This organism is the primary cause of the South African tick fever (Tete fever), so-called because the carrier is a species of cattle tick (*Ornithodoras moubata*).

3. *Spirochæta novyi.*—Said to be the cause of American relapsing fever.

4. *Spirochæta vincenti.*—Pathogenic; causes throat inflammation (Vincent's angina).

5. *Treponema pallidum.*—The specific cause of syphilis. Often other related organisms are found associated with it. This organism stains with difficulty.

6. *Trypanosoma gambiense.*—This is the cause of the dread sleeping sickness of Africa. The transmitter of the infection is the tsetse fly

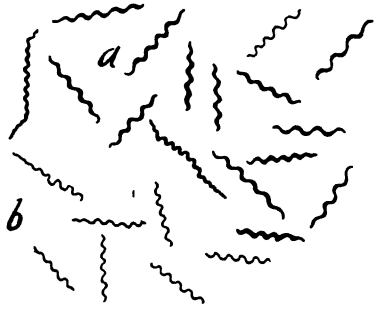


FIG. 74.—*A, Spirocheta refringens; b, Spirocheta pallida.* The cause of syphilis.

(*Glossina palpalis*). Investigations have shown that the eradication of the tsetse fly would also eradicate the disease (Koch), which has practically depopulated large districts in Africa. Related organisms cause diseases in horses (surra, dourine and mal de caderas). There are also many trypanosomes of frogs, fish and birds, but these are probably harmless to man.

Species of *Leishmania* cause sores and ulcers (in tropical countries). Certain tropical *Lamblia* and *Trichomonas* species may cause intestinal and other disturbances.

The infusoria proper (Ciliata), while exceedingly abundant and widely disseminated, are mostly non-pathogenic. The *Balantidium coli* is a common hog parasite which may also cause serious dysentery in man.

III. SPOROZOA.—Commonly designated as amœbæ. Have no cilia, move by plasmic contraction of the cell and reproduce by spores. Of this group, the most important species is the *Plasmodium malariæ* which is the primary

cause of ague or malaria. The carriers of the infection are certain mosquitos (species of *Anopheles*). If the Anopheles group of mosquito could be exterminated throughout the world, malaria would disappear also. The organism is introduced into the blood by the sting of the insect. In the blood it undergoes certain cycles of development. The fever paroxysms are due to the sporulation of the organisms in the circulatory system. During the intervals (non-sporulation) there is no marked febrile disturbance. There are several species of Plasmodium causing the several forms of malaria. The benign tertian (*P. vivax*) has a cycle of forty-eight hours; the quartan (*P. malariæ*) has a cycle of seventy-two hours; and the malignant tertian (*P. falciparum*) has a cycle of forty-eight hours. In the latter type the paroxysms are so severe as to give rise to a continued fever. Quinine is fatal to the Plasmodium and this remedy should be given as a prophylactic and as a cure.

The draining of swamps and other breeding places for mosquitos has reduced malaria. The use of mosquito netting, screens, etc., has also checked this disease. Small water areas may be treated with crude petroleum oil which kills the mosquito larvæ.

The primary cause of yellow fever is as yet unknown but it has been definitely determined that the carrier is a mosquito, the *Stegomyia calopus*. Yellow fever is essentially a tropical disease, though it may flourish in temperate zones until checked by frost which is so readily fatal to the carrier, the mosquito. It has been ascertained that the *Stegomyia* does not occur far from human habitations, that it breeds generally in barrels and cisterns containing rain water, rather than in ponds or larger bodies of water, more remote from habitations. These discoveries have made possible a very effectual campaign against this dread disease. The Federal Government aided by State and Local Boards of Health have insisted on a discontinuance of those breeding places which can be controlled easily. The larger more public breeding places were covered with crude oil. Screening windows and doors and sulphur or Pyrethrum fumigation of mosquito-infested houses and rooms was insisted upon and individuals were instructed in methods of self-protection against the bites of mosquitos. As a result the yellow fever ravages are now reduced to a minimum.

## CHAPTER XI.

## DISINFECTANTS AND DISINFECTION. FOOD PRESERVATIVES. INSECTICIDES.

The pharmacist should be well informed regarding disinfectants and their uses in order that he may assist physicians and health officers in carrying out sanitary rules and regulations in which disinfectants play so important a part. The pharmacist should know how to disinfect sick rooms, private homes and public buildings. He should in addition be informed regarding the essentials in the construction of sanitary homes, shops and stores. He should be able to give good advice regarding water supply, sewage disposal and on preventive medicine generally. He should be well informed regarding the preservation of foods, the use and abuse of food preservatives and on food adulteration and should be prepared to test foods as well as drugs as to quality and purity. He should be informed regarding the nature and use of insecticides and pest exterminators generally.

*Disinfectant* is synonymous with germicide and means any substance, usually in the form of a liquid or gas, capable of destroying bacteria and their spores, more particularly the pathogenic forms. A septic substance is one contaminated or infected with pathogenic or otherwise objectionable bacteria. An aseptic substance is one free from bacterial infection or contamination, but not necessarily possessed of disinfecting or even preserving power. More broadly speaking, disinfectant means any ponderable or imponderable agent or substance, destructive to bacterial life and it is in this sense that the term is here used. Preservatives may be defined as mild disinfectants; that is, when used in larger amounts or stronger concentration, preservatives become disinfectants. Furthermore, the term preservative usually applies to substances added to foods for the purpose of preventing or retarding microbic infection and microbic development. It is, however, also applied to other substances. We speak, for example, of wood preservatives, leather preservatives, fur preservatives, etc., meaning thereby substances which will prevent certain decomposition or other destructive changes in the articles named, due to a variety of organisms as mould, larvæ, insects, mites, etc.

The chief purpose in disinfection is to check and prevent the spread of communicable diseases, by destroying the primary causes thereof, namely, the pathogenic bacteria or other disease producing organisms. The agents or

substances which have disinfecting powers or properties are legion. We can only refer to a few of the more important ones, those which are commonly employed, giving the methods of their use and explaining their action.

Disinfectants differ greatly as to germ destroying powers and attempts have been made from time to time to standardize them or, in other words, to determine their comparative germicidal efficiency, but thus far no satisfactory or generally acceptable method has been adopted. All methods appear to have some objectionable features. The technic and principles involved in the standardization of antiseptics include the following:

1. Selecting some antiseptic as the unit of comparison, as a 1 per cent. solution of phenol.

2. As test objects, definite quantities of bacterial cultures are used; as bouillon cultures of the typhoid bacillus, colon bacillus, hay bacillus, etc. Some experimenters first air dry the bacteria before exposing them to the disinfectants to be tested. There are a number of methods known as the "silk-thread method," "the glass-rod method," "the platinum-loop method," "the spoon method," and others.

3. Exposing the bacteria from a standard culture, for definite periods (uniform for the series of tests) of time, to varying strengths of the disinfectants to be tested.

4. Plating out (in Petri dishes) the exposed bacteria in order that the death point may be ascertained.

The results are expressed numerically by dividing the strength of the disinfectant tested which will kill a given organism in a given time by the strength of the phenol solution which under the same conditions will kill the same organism in the same time. To illustrate, we will suppose that a 1–40 solution of formaldehyde will kill the pest bacillus in ten minutes at 37° C. and that a 1–110 solution of phenol will kill the same organism in the same length of time and at the same temperature, then we get as the phenol coefficient of formaldehyde, 0.36 (40/110=0.36), which means that formalin is only about one-third as active as phenol as far as the destruction of the pest bacillus is concerned. The phenol coefficient is also known as the Rideal-Walker (R-W) coefficient, named after the English investigators who worked out the details of the method. In time no doubt an international standard method for testing disinfectants will be adopted. This would be of inestimable value for comparative purposes.

## 1. Physical and Mechanical Disinfectants.

The following is an outline of the physical and mechanical means of disinfecting.

A. *Cleanliness.*—That is, bacterial cleanliness, or absence of bacteria,

brought about in a variety of ways. The liberal use of pure water for washing, bathing and cleansing purposes is one of the oldest methods for getting rid of pathogenic and otherwise objectionable organisms. It is, at the present time, one of the most effectual means of disinfection, practised by the housewife, the nurse, the physician, in fact by all classes and conditions of peoples. By bacterial clleanliness we bring about a dilution, an attenuation, a dissemination of objectionable organisms to such a degree that bacterial localization and infection are greatly retarded or are made impossible. Cleanliness prevents filth and dirt accumulation.

B. *Pure Air.*—Pure air, that is air free from disease organisms, is a prime essential in preventive medicine. Not only should the air we breathe, be free from bacterial infection, but it should also be free from smoke, fumes, noxious gases, soot and dust. The air in many of our large cities is often quite unsuitable for breathing purposes due to fumes, soot and smoke from numerous furnaces and factories, stenches from sewage, from stock yards, from gas factories, etc. This should not be. Stock yards, glue factories, etc., should be sufficiently remote from cities to avoid permeating the city with the horrible stenches emanating therefrom. Smoke, fumes and noxious gases should not be permitted to escape. The recent tests with smoke consumers, with the precipitation of fumes and smoke by means of electricity, etc., would indicate that it is possible to prevent the pollution of the atmosphere by the above agents. Just as soon as there is a smoke consumer on the market that is a practical success, every smoke producing furnace should be supplied with one, irrespective of cost. The same should apply to the use of smelter fume precepitators. Streets should be kept comparatively clean from dirt and dust by means of sprinkling cart and street sweepers and cleaners.

The "no spitting" ordinances are largely a failure simply because no provision is made to supply the appurtenances necessary to carry them out. It is not sufficient to simply put up a notice stating that "It is unlawful to spit upon the floor," but cuspidors, or other receptacles must be provided in sufficient numbers, conveniently placed, and furthermore said receptacles must be kept clean and sterilized from time to time, otherwise they may become the breeding places and disseminators of disease.

A most serious defect in places of habitation is the lack of pure air, as in small bed-rooms, in the Pullman sleepers, in sweat shops, in factories, in school-rooms. Next to the crowded sweat shops in our large cities, the lower berth in the American Pullman car, is most unsuitable for human habitation. Rooms for living purposes, sleeping purposes, for factory use, office use, etc., etc., should not only be large enough, but there should be adequate provision to renew the air constantly, no matter how warm or how cold it may be. We need a thorough sanitary supervision of all building

construction whether private home, school, factory, sleeping car, office, or street car. There is plenty of pure air and every individual should have an ample supply, for pure air is one of the most potent factors in preventive medicine.

C. *Heat.*—Heat is one of the best disinfectants known. Dry and moist heat are used, both of which have been sufficiently treated in the preceding chapters. Mere dryness is in itself a germ destroyer. Microbes require moisture for their growth. Most bacteria (vegetative cells, not spores) succumb in a dry atmosphere in a comparatively short time, several hours to several days. The spores may, however, survive dryness for many months.

The dry-air temperature usually employed for germicidal purposes, ranges from 100° C. to 150° C., acting for one hour or longer. A dry heat of 145° C. acting for one hour is sufficient to kill all bacteria, including the spores. Temperatures used for purposes of disinfection and sterilization range from 60° C. to 120° C. 60° to 75°C. is usually employed in the pasteurization of milk and in sterilizing sera, vaccines, certain culture media (as egg albumen, blood serum), etc. Moist heat of 100° C. in the form of circulating steam vapor is much used. To obtain a moist temperature above 100° C., an autoclave is necessary, or liquids may be employed which boil at a temperature higher than 100° C. as cumene.

D. *Cold.*—Cold, 10° C. and lower, has decided aseptic properties, that is, it checks bacterial growth and activity very effectually, as has already been explained. Prolonged freezing is, however, necessary to kill bacteria. Cold may therefore be considered a most excellent check upon bacterial activity, but it is a very poor germicide. Cold is a universally recognized and an extensively used food preservative, due to its checking influence upon bacterial growth.

E. *Agitation.*—The agitation of gases and liquids reduces the bacterial activity therein. Still waters become stagnant but running waters do not, in the comparative sense, due in part to the difference in the oxygen content. Agitating and churning contaminated liquids checks bacterial development somewhat. The active circulation of contaminated air reduces the number of bacteria present. Agitation is, however, not a satisfactory means of sterilization and disinfection.

F. *Sedimentation and Filtration.*—Sedimentation in sewage waters and other contaminated liquids, combined with filtration, is a very effectual means of purification. Precipitation and filtration, aided by chemicals as alum, iron sulphate, and other coagulants, are much employed in the purification of water supplies.

G. *Free Circulation.*—Free circulation of air and water are most favorable to sanitation because of the checking influence upon bacterial activity and also because of the disseminating and diluting effects upon the organisms

which may be present. Circulation is strictly speaking a means of cleansing.

Purification of flowing water, as rivers and small streams, is effected very largely by oxidation and dilution. The agitated water takes up oxygen by absorption which combines with the organic particles suspended in the water rendering it unsuitable as food for bacteria. As the water flows along the bacteria are scattered more and more. Sedimentation is also an important factor in the destruction of bacteria. Gradually the bacteria settle to the bottom of the stream where they are brought in competition with other bacteria, protozoa, algæ, perhaps hyphal fungi, etc., which tend to check and even entirely inhibit their further development.

H. *Light.*—Sunlight has most marked germicidal powers, due in part, to the drying effects produced and in part to the actinic or chemically active rays of the sun's light. Numerous investigators have demonstrated the germ-destroying effects of the blue and violet-rays and the ultra violet end of the solar spectrum. Bacteria cannot survive in sunlight. Electric light is said to have the same effect upon bacterial life as sunlight. The *X*-rays destroy bacteria, likewise does radium, and these agents have been extensively tested in the treatment of skin diseases and superficial tuberculosis as lupus, and in cancer, but without satisfactory or conclusive results. The germ-destroying effects of sunlight are not due to heat as may be shown by the use of an alumn tank which intercepts the heat rays.

I. *Electricity.*—The electrical current in itself appears to be without germicidal powers, but electricity is used to precipitate smelter fumes, and organic impurities in water, as already stated. Electricity is used to stimulate seed germination and it may be possible to utilize electrical discharges or currents in the treatment of communicable diseases.

## 2. Chemical Disinfectants.

Chemical disinfectants may be divided into gaseous (or vaporous) and liquid (solutions). The liquid disinfectants are superior to the gaseous disinfectants because direct contact with the articles to be disinfected can be brought about, as in washing, immersing or mixing. Gaseous disinfectants are effective for surface sterilization, especially useful for inaccessible rooms, buildings, ships, paintings, books, fabric, etc. Both have their special advantages, however.

The number of chemical disinfectants, variously classed as gaseous, liquid, patent, proprietary, efficient, useful, useless, etc., etc., is very great. We shall mention only a few of the more powerful kinds. No reliance should be placed in any patented or proprietary disinfectant until its value has been demonstrated by tests made by reliable bacteriologists, giving its phenol coefficient. Nor is this all, not only must the disinfectant have actual germ-

destroying powers, but it must also be practically usable and it must not be misrepresented as to its value and its application and use in practice.

The resistance of pathogenic germs to disinfectants is extremely variable. Furthermore, the various disinfectants produce changes in the tissues and substances in and upon which they act, which changes tend to modify, check or inhibit the disinfecting powers. Thus a number of disinfectants may have the same laboratory phenol coefficient and yet their value as disinfectants in actual practice is widely different because of the difference in the effects produced in and upon the substances with which they are brought in contact.

As a rule, the action and use of disinfectants is variable according to the following conditions:

1. Disinfectants are more active when warm or hot. In all disinfections hot solutions should be used, if possible and if practicable.

2. Gaseous disinfectants act only in the presence of moisture, as will be explained under formalin and sulphur disinfection.

3. The thoroughness of disinfection is directly proportional to the time that the disinfectants are allowed to act.

4. The activity of disinfectants is directly proportional to the degree of concentration, though there are noteworthy exceptions. Absolute alcohol, for example, is of very little value as a disinfectant, whereas the weaker solutions (40 to 70 per cent.) are a very active germ destroyer. The same is true of ether, chloroform, glycerin and a number of other substances. Most disinfectants have a concentration of optimum or maximum efficiency which is the degree of concentration generally employed in practice.

5. In actual practice the cost of disinfectants is a very important desideratum, as is indicated by the following table giving the comparative phenol coefficient and the relative cost:

| Name of Antiseptic | Phenol Coefficient | Comparative Cost |
|---|---|---|
| Carbolic acid | 1.00 | $ 0.25 |
| Chinosol (potassium salt) | 0.03 | 127.87 |
| Condy's fluid (permanganates) | 0.90 | 2.00 |
| Cyllin (a cresol) | 1.00 | 0.08 |
| Formaldehyde | 0.30 | 4.40 |
| Izal (rich in cresols) | 8.00 | 0.12 |
| Listerine (a compound) | 0.03 | 324.62 |
| Lysoform (formalin soap) | 0.10 | 36.49 |
| Pearson's antiseptic (a cresol) | 1.40 | 0.42 |
| Sanitas (contains oil of turpentine) | 0.02 | 42.56 |

6. It is known that the disinfecting power of metallic salts is proportionate to their electric dissociation, that is, the more strongly a salt is dissociated by electrolysis the stronger is its disinfecting power. It follows that anything which interferes with the electrolytical dissociation of germicides weakens the germicidal power. For example, the addition of sodium chloride lowers the germ destroying powers of corrosive sublimate through such interference. This is a matter of great importance in determining the value of antiseptics.

7. The chemical composition of the material associated with the germs to be destroyed has a marked influence upon the action of the germicides. Thus germicides give different results when acting upon the same organism in water, in beef broth, in salt solutions, in and upon tissues, etc. For this reason the value of germicides in actual practice cannot be based exactly upon the laboratory results.

8. Not only do different species of disease germs differ in resistance to germicides, but the different strains of the same species react differently with the same germicide. Certain organisms appear to have an elective affinity for certain chemicals, as for example, the malaria germ for quinine, and the syphilis germ for mercury salts.

Disinfectants destroy or kill germs in different ways. In some cases the death of the organism is due to oxidation as when ozone, hydrogen peroxide and sulphites are used, or death may be due to interference with nutrition, but more generally it is due to the coagulation of albumen and abstraction of water from the cell-plasm, as in the use of dry heat, phenol, alcohol, tannic acid and metallic salts. As already explained in another chapter, lysins act by actually disintegrating the bacterial cells.

The action of disinfectants depends upon a great variety of conditions, entirely too numerous and too complicated to be fully treated in a text-book, nor is it necessary to enter into lengthy discussions and explanations. In time many of the points still in dispute will be definitely worked out, in the laboratory.

As a rule germicides are most active when dissolved in water, though some authorities declare that bichoride of mercury, phenol, thymol and lysol are more active when dissolved in 50 per cent. alcohol. The activity of phenol as a germicide is greatly increased by the addition of hydrochloric acid, whereas lime reduces its potency. Solutions of germicides in oils are inert because oil does not penetrate the bacterial cell; however, the oil itself may be fatal to bacterial life, in which case the added germicide is unnecessary. Chemical germicides do, however. increase the potency of the volatile coal-tar products as gasoline, benzine and xylol, provided the germicides are soluble in these substances.

The following are the more important disinfectants given approximately in the order of their usefulness and potency.

A. *Carbolic Acid (Phenol).*—Very widely used, in strengths of from 1 to 5 per cent. As a disinfecting wash for all manner of septic things, a 5 per cent. solution is commonly employed. A 2.5 per cent. (also the 5 per cent.) solution is much used as a disinfectant for hands and the skin generally and for septic wound irrigation. A 0.5 to 1 per cent. solution is used as a mouth wash and gargle. Phenol does not kill spores hence should not be used after anthrax, tetanus, malignant edema and other diseases due to spore bearing bacteria. Phenol coagulates albumen, but not as actively as does corrosive sublimate.

Carbolic acid (5 per cent.) is much used for disinfecting liquid discharges in dysentery, typhoid, cholera, and for the disinfection of sputa and expectorations in tuberculosis, in pneumonia, etc., using about two times as much of the disinfectant as material to be disinfected, allowing the mixture to stand for several hours at least.

A 5 per cent. solution may be prepared as follows:

| | |
|---|---|
| Carbolic acid (95 per cent.), | 6 1/2 oz. |
| Water, | 1 gal. |

Shake thoroughly until all of the acid is dissolved.

Carbolic acid does not destroy, bleach or discolor cloth fabric, does not corrode metal, has a marked characteristic odor, is a powerful escharotic poison, and the crystals are readily liquefied by heat, by alcohol and by water.

B. *Liquor Cresolis Compositus U. S. P.*—This most efficient germicide is a liquid soap with 50 per cent. cresol, miscible in all proportions with water. The cresols used should have a high boiling-point (187° to 189° C.). The germicidal powers of this substance is nearly double that of carbolic acid. It does not coagulate albuminous matter and kills spores.

There are a number of germicides similar to carbolic acid having marked germicidal properties including creolin, cresol and lysol. These are somewhat superior to carbolic acid. Lysol is a cresol mixed with soap which greatly facilitates the solution of the cresol, being therefore similar to liq. cres. comp. U. S. P. They all kill spores.

C. *Tricresol.*—Tricresol is a mixture of orthocresol, metacresol and paracresol. It dissolves in water in the proportion of 2.5 per cent. and is about three times as active as carbolic acid. It is less irritating than carbolic acid for which reason it is preferred in sterilizing sera (about 0.25 per cent.) and other solutions intended for hypodermic use. Tricresol kills spores and albuminous matter does not interfere with its action.

Tricresol, cresol, lysol, solveol, solutol and creolin are usually employed (as germicides) in 1 per cent. solutions and are generally conceded to be equal to about 2.5 per cent. solutions of phenol. They, however, have no superiority over the liq. cres. comp. U. S. P.

D. *Formalin.*—The 40 per cent. commercial article is used. It has

many advantages as a disinfectant. It does not injure, fade or decolorize cloth or other colored fabric and does not corrode metal (excepting hot steel and iron). It kills spores and is an efficient deodorant. Albuminous matter does not interfere with its action and hence it is an efficient sick-room disinfectant. It disinfects and deodorizes all discharges from patients very quickly and completely, when used in 4 to 5 per cent. solutions.

As a gaseous disinfectant it is active in a moist, warm atmosphere. It does, however, not kill insects and other higher organisms and in this regard it is inferior to sulphur dioxide, but has the advantages of not decolorizing fabric and being a better deodorant. There are several proprietary disinfectants composed of soap and formalin, as lysoform.

E. *Sulphur.*—Sulphur in itself is odorless, tasteless and wholly inert as a germicide, but when undergoing oxidation into sulphur dioxide (combustion), in the presence of moisture, it is a very active disinfectant and is at the same time fatal to insects and in fact to all forms of animal life, including rats, mice, etc. But it cannot be used to disinfect fine fabrics, paintings, books, etc., because of the destructive effects upon pigments.

Under ordinary conditions the gaseous substances, as formaldehyde (formalin) and sulphur dioxide, are surface disinfectants only and are used where surface disinfection is all that is required, as in the sterilization of clothing, wood work, walls, ceilings, pictures, furniture, etc.

F. *Bichloride of Mercury.*—This is the most potent and most extensively used of all antiseptics. A 1–1000 aqueous solution (used hot whenever and wherever possible) makes a most satisfactory germicidal wash for floors, walls, wood work of all kinds, in fact anything requiring disinfection, excepting metals which would be corroded (excepting of course platinum, gold, silver) and substances rich in albuminous matter as pus, sputum, and other sick room discharges, which are coagulated by this germicide, checking further action.

The 1–1000 solution is sufficiently powerful to kill all non-sporogenous bacteria at the ordinary room temperature in one-half hour. For spores a stronger solution (1–500) and longer exposure are desirable (one hour).

The chief disadvantages to the use of corrosive sublimate are its highly toxic nature, its corroding effect upon metals and its coagulating effects upon albumen which hinders penetration. It should also be borne in mind that soap interferes with the action of corrosive sublimate.

A 1–1000 solution is made as follows:

| | |
|---|---|
| Bichloride of mercury, | 61 1/2 grs. |
| Citric acid or salt, | 61 1/2 grs. |
| Water, | 1 gal. |

The salt or citric acid is added to retard the decomposition of the bichloride. Tablets are now on the market prepared from mercury cyanide.

They are held to be more decidedly antiseptic than either the iodide or the bichlorid of mercury, and are so prepared that one tablet added to a pint of water will make a strength of 1–1000.

G. *Chlorinated Lime.*—Also known as chloride of lime. This is an oxidizing disinfectant and deodorant, most extensively employed for the disinfection of stools, urine, sputa and other excreta. Eight ounces of the chlorinated lime are added to one gallon of water. This solution is placed in the vessel which is to receive the discharges, using at least double the amount to be disinfected and allowing the mixture to stand for one-half hour or longer. Chlorinated lime destroys color and corrodes all textile fabrics and most metals. It must be kept in an air tight receptacle as it loses in strength on exposure to air. The solutions should be made as required.

H. *Lime.*—Lime (unslaked lime, quicklime) is very useful for the destructive disinfection of cadavers dead of infectious diseases, using twice the amount of lime, by weight, to the substance to be disinfected. The lime is powdered or crushed and packed about the cadaver in a box or coffin. Neither water nor moisture need be added.

I. *Milk of Lime.*—Lime is slaked in the usual way. From the slaked lime the milk of lime is prepared by adding eight parts of water. The preparation should always be made from freshly slaked lime. It is much used for the disinfection of stools and sputum, using an amount equal to the amount of material to be disinfected. Whitewash is much used to disinfect and preserve fences, stables, sheds, walls, ceilings, etc.

J. *Copper Sulphate.*—Blue vitriol is a very useful disinfectant for sick room excreta of all kinds, using a 5 or 10 per cent. solution, bulk equal to bulk of material to be disinfected, stirring and mixing and allowing to stand for 3 to 4 hours. Iron sulphate (copperas) is similarly used, though it is somewhat weaker in action.

K. *Permanganate of Potassium.*—This is another of the oxidizing antiseptics, having a rather limited use. It is furthermore comparatively expensive. Freshly prepared solutions are used, ranging in strength from 1–1000 up to 5 per cent. Quite extensively used as a disinfectant for hands. Has been administered internally to oxidize alkaloidal poisons in the stomach and in the intestinal tract.

The following antiseptics are used more or less in surgery and as skin and other tissue disinfectants. Some of them are used as general disinfectants, but as a rule they are not sufficiently powerful to be of much practical value.

A. *Iodoform.*—Formerly much used as a dressing for syphilitic ulcers. It is not germicidal but has decided aseptic and sedative properties hence also used in scalds and burns. It may, however, cause dermatitis. It is insoluble in water but freely soluble in ether and alcohol. The ointment (containing 10 per cent. iodoform) is still much used. Aristol, europhen

iodol, losophen and nosophen are iodoform derivatives, have similar properties, less odorous, less irritating and less poisonous. The persistent disagreeable odor of iodoform is a great objection to its use.

B. *Boric Acid and Borax.*—Boric acid is a very mild antiseptic and hence is of little or no practical value as a germicide but it is an ideal aseptic agent. It can be applied to comparatively aseptic cuts, bruises, wounds, etc., in saturated solution (aqueous) or in powder. It can be applied as a dusting powder to many conditions where an aseptic substance is indicated. In saturated solution it makes a good gargle, mouth wash, eye wash, etc.

Borax is similarly used and has similar properties. The choice between the two is decided by the difference in reaction. Boric acid is slightly acid in reaction, whereas borax is slightly alkaline. The preparation boroglycerin is much used as a dressing for inflamed and infected mucous membranes.

Sixteen grains of salicylic acid and 96 grains of boric acid dissolved in a pint of sterile water makes Thiersh's fluid. This is useful in cleansing mucous membranes, such as those of the mouth and eye, and it may be used in the form of irrigations for cleansing purposes.

C. *Creosote.*—This excellent germicide is rarely used for general external disinfection though it is more active than phenol and does not coagulate albumen and is less toxic and less irritating. In doses of from 1 to 10 minims (given internally) it is much used as an antiseptic and stimulant in tuberculosis and to correct intestinal fermentation. The carbonate of creosote is said to be especially efficacious in lung troubles (tuberculosis). Creosote is essentially an intestinal antiseptic.

D. *Hydrogen Dioxide.*—This is the most active of the oxidizing disinfectants, used in solutions of from 10 to 15 per cent. It is a very active bleaching and deodorizing agent. It is not used for general disinfection but is one of the best known local germicides, applied to abscesses, ulcers, used as a spray, as a gargle, etc. Much employed in dental work. Used by bacteriologists to determine the amount of bacteria in milk (indicated by gas liberation when added to the milk in fermentation tubes).

E. *Naphthalene Derivatives.*—These are used as intestinal antiseptics but are of doubtful value in the treatment of intestinal diseases. They are not acted upon in the stomach secretions but on reaching the intestinal tract they undergo a chemical change and act as antiseptics. Their prolonged use produces irritation of intestines, bladder and kidneys. To this group belong betanaphthol, betol, naphthol naphthalin, and others.

To the group of so-called intestinal antiseptics belong antipyrin, acetanilid, phenacetin, phenecol, quinine, salicylic acid, salol, salophen, guaiacol, resorcin and many other substances. Their value as intestinal antiseptics is very problematical and doubtful.

F. *So-called Respiratory Antiseptics.*—There are a great variety of volatile or gaseous substances which are said to act as antiseptics to the respiratory tract when inhaled, as oil of thyme, eucalyptol, oil of eucalyptus, menthol, camphor, euthymol, campho-phenique, mint oil, etc., but their value in this regard is *nil.* They may have some stimulating effect upon the tissues of the respiratory tract but they do not destroy any germs which may be present upon or within the cells of the respiratory passages.

The following table taken from the work by Ellis gives the minimum proportion of germicidal activity of well-known disinfectants. The figures indicate the strength of solution necessary to prevent bacterial development when added to substances capable of giving rise to bacterial growth, naming therefore the aseptic strength and not the actively antiseptic strength. The figures are not absolute for reasons which have been fully set forth in the beginning of this chapter. The table is merely a guide to the relative activity of the germicides named.

1. *Very active antiseptics.*

| | |
|---|---|
| Mercuric iodide, | 1–40000 |
| Silver iodide, | 1–33000 |
| Hydrogen peroxide, | 1–20000 |
| Mercuric chloride, | 1–14300 |
| Silver nitrate, | 1–12500 |

2. *Active antiseptics.*

| | |
|---|---|
| Osmic acid, | 1–6666 |
| Chromic acid, | 1–5000 |
| Chlorine, | 1–4000 |
| Iodine, | 1–4000 |
| Chloride of gold, | 1–4000 |
| Bichloride of platinum, | 1–3333 |
| Hydrocyanic acid, | 1–2500 |
| Bromine, | 1–1666 |
| Copper chloride, | 1–1428 |
| Thymol, | 1–1340 |
| Copper sulphate, | 1–1111 |
| Salicylic acid, | 1–1000 |

3. *Fair antiseptics*

| | |
|---|---|
| Potassium bichromate | 1–909 |
| Potassium cyanide, | 1–909 |
| Ammonia, | 1–714 |
| Zinc chloride, | 1–526 |
| Mineral acids, | 1–500 |
| Lead chloride, | 1–500 |
| Nitrate of cobalt, | 1–500 |
| Carbolic acid, | 1–333 |
| Potassium permanganate, | 1–285 |

| | |
|---|---|
| Lead nitrate, | 1–277 |
| Alum, | 1–222 |
| Tannin, | 1–207 |

4. *Indifferent antiseptics.*

| | |
|---|---|
| Arsenious acid, | 1–166 |
| Boric acid, | 1–143 |
| Arsenite of soda, | 1–111 |
| Hydrate of chloral, | 1–107 |
| Salicylate of soda, | 1–100 |
| Iron sulphate, | 1– 90 |
| Caustic acid, | 1– 56 |

5. *Feeble antiseptics.*

| | |
|---|---|
| Calcium chloride, | 1–25 |
| Sodium borate, | 1–14 |
| Alcohol, | 1–10 |

6. *Very feeble antiseptics.*

| | |
|---|---|
| Ammonium chloride, | 1–9 |
| Potassium iodide, | 1–7 |
| Sodium chloride, | 1–6 |
| Glycerin, | 1–4 |
| Ammonium sulphate, | 1–4 |

### 3. Procedures for Disinfection.

Space will not permit entering into a full discussion, nor is this necessary as it may be presumed that the pharmacist will know how to meet the special conditions which may arise, should he be called upon to do so.

A. *Surgical Disinfection.*—a. The operating room must be clean and free from pathogenic and other objectionable organisms. The room must therefore be disinfected from time to time, after the method of procedure for any room which may be assumed to be infected, as will be explained under room and house disinfection. As to when, how often or how completely the operating room is to be disinfected that must be left to the judgment of the surgeon in charge.

b. Surgeons should be especially careful regarding personal cleanliness, irrespective of the routine personal disinfection and sterilization performed preparatory to an operation. They should always be smooth-shaven as the beard is a carrier of germs.

c. On preparing for an operation the surgeon removes coat, cuffs and collar in an ante-room; rolls up shirt sleeves and proceeds to wash and scrub hands with tincture of green soap, then in 1–1.5 per cent. tinct. cres. comp. U. S. P. or lysol, rinse in sterile water, dry with a clean sterile towel and dip in 50 to 60 per cent. alcohol. Formalin and carbolic acid should not be used

as hand disinfectants (by the surgeon) because of the benumbing effects of these chemicals, causing a lessening in the delicacy of touch. A 1 per cent. solution of potassium permanganate is recommended as a disinfectant for hands. In many hospitals nothing more than a thorough scrubbing with green soap is employed for the hands of surgeons, with wholly satisfactory results.

Before entering the operating room the surgeon and attendants don sterilized gowns with hoods covering head, hair, and face (beard), leaving only the mouth, nose and eyes free. The hands of the attendants are covered with sterilized rubber gloves.

d. The surgical instruments are washed and wiped dry; boiled for ten minutes, in water with 1 per cent. soda, and laid in a tray containing 5 per cent. carbolic acid solution. Before using, they are rinsed in boiled distilled water. Never sterilize metallic instruments in corrosive sublimate, or in any corrosive disinfectants of any kind. Only a short exposure would suffice to dull the keen edge of knives, scalpels, and other cutting instruments. Do not sterilize steel instruments in hot air as high temperatures reduce the temper, and do not sterilize them with rubber goods.

B. *Sick Room Disinfection.*—Disinfection in the sick room of a patient afflicted with some communicable disease, may be divided into disinfection of dejecta, urine and sputum; disinfection of the patient; disinfection of clothing and bedding; disinfection of the sick room itself; and precautionary disinfection of the attending physicians, nurses and attendants. In case of fatal termination of the malady there is included disinfection after post-mortem and sterilization of the dead body. In all cases, whether the patient dies or recovers, the entire sick room, including bed, chairs, bedding, etc., must be thoroughly disinfected. The methods of procedure may be outlined as follows:

a. *Disinfection of Excreta.*—To disinfect dejecta, urine and sputum, a 4 per cent. solution of chloride of lime or a 20 per cent. solution of milk of lime will be found very efficient, using amounts of the disinfectants equal to the bulk of the excreta to be disinfected, mixing well and allowing to stand for one hour. The disinfectants are first placed in the vessels intended to receive the excreta, more being added afterward if it is thought desirable. If sputa and other excreta are received upon napkins or other cloth, these should be burnt at once, or if that is not convenient they may be placed (entirely immersed) in the disinfectant. For tuberculous sputum the chloride of lime is best. Spit cups should be kept two-thirds full of the 4 per cent. solution. Paper spit cups are to be destroyed by burning as soon as possible.

Sulphate of copper (5 to 10 per cent. solution), or carbo-hydrochloric acid solution (5 per cent. each of phenol and hydrochloric acid) may be used in place of the chloride of lime and milk of lime. Bichloride of mercury

and phenol (without the hydrochloric acid) are not very satisfactory for disinfecting excreta because of the coagulating effects upon albuminous matter. Liquor cres. comp. U. S. P., lysol and tricresol (2–2.5 per cent. solutions) may be used. Weak disinfectants or untried patent or proprietary disinfectants should never be used for above purposes. For example, permanganate of potassium, boric acid, borax, glycothymoline, borol, etc., would be valueless as disinfectants for excreta.

b. *Disinfection of Patient.*—This includes cleaning the body surface with soap and water, with 50 to 70 per cent. alcohol, washing with 1 to 2.5 per cent. solutions of phenol, cres. comp., lysol or creolin, when so ordered by the attending physician. Bichloride of mercury (1–2000 to 1–1000) may be used for skin disinfection. A saturated solution of boric acid, normal salt solution or a 1–1000 solution of permanganate of potassium may be used as a wash or irrigation for non-infected wounds and cuts, etc., but not for ulcers, abscesses, etc.

Irritating disinfectants should not, for very obvious reasons, be used. In every case the mode of procedure in the disinfection of the patient will be outlined by the attending physician.

Nurses, attendants and physicians must observe the necessary precautions against becoming disseminators of the infection and must resort to certain methods of self disinfection after each visit to the patient, as in small-pox, plague, diphtheria and other communicable diseases.

c. *Disinfection of the Clothing Worn by the Patient and of the Bedding.*—All clothing worn by the patient and all bedding, as soon as ordered changed, should at once be immersed in a hot, 5 per cent. solution of carbolic acid or a 2.5 per cent. solution of cres. comp. or lysol. After soaking for several hours the clothing should be boiled in water for 30 minutes at least. After thorough drying, preferably in the sun, the clothing should be well ironed. The ironing process in itself has very marked germicidal powers. Clothing may also be disinfected in formalin (4 per cent.). Sulphate of copper and sulphate of iron discolor and corrode the cloth. All cloth fabrics and clothing which has been in close contact with a patient suffering from diphtheria, cholera, plague or small-pox, should be destroyed by burning whenever possible.

d. *Disinfection of the Sick Room.*—The bed frame, the chairs and other wooden furniture, the floor and the wood work of the room, may be washed or wiped with corrosive sublimate (1–1000), formalin (3–4 per cent.) or phenol (5 per cent.), if contamination is suspected or if so ordered by the physician, even while the room is still occupied by the patient.

Just as soon as the patient is taken from the room, a thorough disinfection should be carried out at once, the disinfection including furniture, clothing of the patient, bedding, mattresses, pillows, etc., excepting such articles as are ordered destroyed by burning.

Every pharmacist should fully inform himself regarding the state laws and city ordinances governing health and quarantine regulations. State and city boards of health usually issue free bulletins on methods of disinfection in communicable diseases. Copies of these should be on hand for ready reference.

For room disinfection, formalin or sulphur are used. With formalin the procedure is as follows: For every 1000 cubic feet of space there is required one pint of formaldehyde (the 40 per cent. commercial formalin) and 8 ounces of commercial potassium permanganate. Place the permanganate in an agate lined or iron pail of about ten times the capacity of the disinfectant to be used, spreading the permanganate evenly over the bottom. Set pail containing the crystals upon a brick, iron stand or other support, in a tub, pan or dish partially filled with water. See that windows and doors are closed and sealed (excepting the exit). The room should be warm and moist, a condition which may be effected by suspending sheets wrung out of hot water about the room. In a steam heated flat, steam may be allowed to escape from the air vent of a radiator, or steam may be generated outside of the room and conducted into it by means of rubber tubing. Do not have an open fire or flame in the room to be disinfected as the gas to be liberated is somewhat inflammable. Having ascertained that all is in readiness, pour the formalin solution from a dipper or wide mouthed vessel over the permanganate; leave the room at once, close and seal exit, plugging key hole and crevices in door. Eighty per cent. of the gas is liberated within ten minutes or less. Leave the room sealed for at least six hours, preferably twelve hours. At the end of this time disinfection is complete. Open doors and windows. Traces of formalin may be destroyed by sprinkling or spraying ammonia in the room.

It is advised to use a separate container for every pint of formalin used. A large piece of matting or other absorptive material may be placed under each container to guard against the possibility of staining the floor, in case the floor requires such protection.

In case sulphur is used, prepare the room (as to sealing, air moisture and warmth) as for formalin disinfection, taking the precaution to remove (and disinfect separately, by means of formalin and bichloride of mercury) paintings, clothing and other fabric which must not be bleached by the sulphurous acid fumes. For every 1000 cubic feet of space use 3.5 pounds of flower of sulphur. Place the sulphur on a bed of sand or on ashes in an iron pot or pan which is supported on a brick or iron stand in a dish of water. Pour a little alcohol over the sulphur and ignite.

Sulphur candles are now found upon the market and are more convenient than sulphur. Place a sufficient number of the candles upon bricks in pans of water and light them. Liquefied sulphur dioxide put up in conven-

ient containers may be employed, using 15 ounces to each 1000 cubic feet. Open the can by means of a can opener, set it in a pan or dish and allow the gas to evaporate.

Remember that the sulphur dioxide corrodes metal, bleaches clothing, hangings and draperies and, with formalin, is without disinfecting power in the absence of moisture.

After the disinfection with formalin or sulphur dioxide is completed, it is often desirable to go over the floors, furniture, bed frames, etc., with a 1–1000 bichloride of mercury solution.

Mattresses, heavy quilts, pillows and furniture cushions are difficult to disinfect with formalin or sulphur dioxide. These should be disinfected by steam under pressure. In such diseases as plague, diphtheria and cholera, such articles should be destroyed by burning. Anyway, a sick room should have simple furniture and merely such articles as are absolutely necessary and such as can be disinfected readily.

The so-called carbo-gasoline method of book disinfection is highly recommended. Immerse books, papers, clothing and other articles to be disinfected for twenty minutes in the carbolized gasoline. Take from the disinfecting solution and allow to dry in the open. The carbolized gasoline consists of Baume 88° gasoline or gas machine gasoline to which 2 per cent. of carbolic acid is added. No injury is done to the books or clothing, provided they are carefully handled until dry. Gasoline will, however, injure oil paint lettering, etc.

D. *Postmortem Disinfection and Sterilization of Cadaver.*—After autopsies on bodies after infectious disease, thorough disinfection must be resorted to. A liberal use of a 4 per cent. solution of calcic hypochlorite, allowing this to act for at least one hour, will serve the purpose.

In cases of death from contagious diseases all orifices of the body should be packed with cotton well soaked in a 1–500 bichloride solution. The entire body should be washed with a 1–1000 bichloride solution. Cremation is desirable and the funeral should be private.

The so-called embalming fluids of funeral directors are aqueous solutions of various chemical disinfectants, having corrosive sublimate and formalin as the chief ingredients. The following formula is said to have the approval of the National Funeral Directors Association of the United States:

| | |
|---|---|
| Formalin (40 per cent.), | 11 lb. |
| Glycerin, | 4 lb. |
| Borax, | 2.5 lb. |
| Boric acid, | 1 lb. |
| Potassium nitrate, | 2.5 lb. |
| Solution of eosin (1 per cent.), | 1 oz. |
| Water, to make | 10 gal. |

The salts are dissolved in six gallons of water; the glycerin, formalin and eosin added and enough water to make up the ten gallons.

E. *Disinfection of Public Buildings and Public Conveyances.*—Only rarely will it become necessary to disinfect an entire large building, whether private or public, and then the method of procedure is much the same as for the sick room disinfection, already described, treating each room as though it were independent of other rooms, excepting that inner connecting rooms need not be closed and sealed.

In disinfection, one important fact should never be lost sight of, namely, that it is just as important to destroy the carriers of disease (flies, fleas, rats, mice, and other animals), as the disease germs themselves. This is particularly important in public disinfection, so much so that it is a general rule to always use a disinfectant which destroys the disease carriers, as sulphur dioxide. In the yellow fever district, for example, the chief fumigating agent is burning Pyrethrum which is a sure death to the Stegomyia mosquito as well as to other insects.

Wherever and whenever practical therefore, sulphur dioxide should be used for public disinfection. In many European cities the health department is provided with portable generators which are run alongside the building to be disinfected, the sulphur dioxide generated and conducted into the room, hall, cellar, or area way to be disinfected, by means of tubing. This is the safest and most satisfactory way. If such apparatus is not available, the flower of sulphur, sulphur candles, or liquefied sulphur dioxide may be used (15 ounces to each 1000 cubic feet of space). Street cars, railway cars, large public conveyances generally, may be disinfected much like rooms, after being well sealed. A safe rule is to use double quantities of the disinfectant for public conveyances, as compared with a sick room, because of the fact that it is difficult to seal such public conveyances well. After the disinfectant has acted for a sufficient length of time (twelve to twenty-four hours), the place is opened, aired and then all of the wood work (of furnishings as well as the floor, walls and ceiling) is either washed or sprayed with a 1–1000 bichloride of mercury solution or a 3–5 per cent. formalin solution.

In such communicable diseases as have no animal carriers (other than the patient himself) or where for obvious reasons such carriers are not present, formalin will always be the preferred disinfectant, whether for private or public disinfection, bearing in mind that heat and moisture are necessary adjuncts to its use. *Formaldehyde is not effective in a dry, cold atmosphere* because under those conditions the formalin is converted into solid polymerized paraformaldehyde, which as such, is inert.

Public or private disinfection by means of formalin may be carried out as follows, the method selected depending upon time, place and opportunity.

a. *Wet Blanket Method.*—Immerse blankets or sheets in the formalin solution and suspend them about the room to be disinfected. The room may first be sprayed with a hot 4 per cent. solution of formalin which furnishes warmth and moisture. The operator must work rapidly as formalin is very irritating to eyes and respiratory tract.

b. *Methyl Alcohol Lamps.*—Formalin may be generated in the space to be disinfected by oxidizing the methyl alcohol and converting it into formaldehyde. Lamps of special construction are necessary. The vapor of methyl alcohol is passed over a highly heated plate whereupon it is oxidized into formaldeyhde ($CH_3OH + O = HCHO + H_2O$) with liberation of water. This method of disinfection is now rarely employed.

c. *Sanitary Construction Company's Lamp.*—The mechanism consists of a tank to hold the formalin, connected with a spiral tube through which the solution is slowly passed through a flame. The heat vaporizes the formalin which is then conducted into the room (through the key hole) by means of suitable tubing. This apparatus is much used by health officers.

d. *The Shering Lamp.*—These small compact and most convenient lamps can be secured from any wholesale drug supply house. With this apparatus the solid tablets of paraform or paraformaldehyde are used. The heat from the lamp decomposes the tablets, producing formaldehyde. The lamps are placed in position, in sufficient numbers, lighted and the small tray of each lamp is supplied with a sufficient number of tablets. As a precautionary measure each lamp should be placed on a brick in a pan or dish of water. The air in the room must be warm and moist.

e. *Formaldehyde Candles.*—These consist of a mixture of paraformaldehyde and paraffin, wax, tallow or other combustible, which may be moulded into candles. The candles are placed in a fireproof dish or pan and ignited. For room disinfection these candles are most convenient as well as satisfactory.

F. *Disinfection at Quarantine Stations.*—All civilized nations maintain a system of vigilance as a protection against the introduction, from foreign countries, of certain communicable diseases designated as quarantinable. The first disease against which a quarantine was established was the plague. In the fourteenth century certain Italian cities established a quarantine against this dread disease and the word "Quarantine" came into general use because of the fact that the period of detention was about forty days (Ital. *quarantina*). The actual period of detention as now enforced varies somewhat depending upon the nature of the disease against which the detention is maintained, as determined by the period of incubation. The quarantinable diseases recognized by the United States are plague (bubonic), small-pox, yellow fever, Asiatic cholera, leprosy and typhus.[1] The enforce-

[1] National quarantine against foreign disease is entirely distinct from state or city quarantine. The following diseases are recognized as quarantinable by most state boards.

ment of the quarantine regulations is under the direction of the Public Health and Marine Hospital Service. The most important quarantine stations in the United States are at San Francisco, New Orleans, New York and Boston, ranking in importance in the order named. The Station at San Francisco is of special importance because upon its efficiency depends very largely the exclusion of plague, cholera and small-pox, the three highly communicable diseases so prevalent in the Orient. Of course a national quarantine to be effective must be complete, covering every port of entry, whether large or small, maritime or inland. This is very often not the case and as a result an epidemic may enter *via* a minor port where the service is inadequate due to incompetent or insufficient inspection.

The quarantine officers are kept informed as to the occurrence of epidemics or sporadic cases of quarantinable diseases in foreign countries and port cities thus putting them on their guard as to the need of special vigilance regarding imports and immigration from such places or cities. However every ship from a foreign port on arriving within the quarantine zone of the station is visited by the boarding officer who immediately proceeds to get data regarding the sanitary conditions on board, as to deaths, sickness of any kind, etc. All passengers, including the ship's crew, are lined up and inspected by the boarding officer. If nothing untoward is reported or detected the captain of the ship is given a clean bill of health and the vessel is permitted to dock and discharge passengers and cargo.

If however the boarding officer finds a case of small-pox or other quarantinable disease on board, the ship is anchored near the station; the passengers and crew are landed at the quarantine station and, with the aid of the ship's officers, the quarantine officer proceeds to disinfect all persons and their personal effects, the same class distinction (first cabin, second cabin, steerage, ship's crew) being maintained as on ship. Each day, as long as the quarantine lasts, all persons are examined by the chief officer of the station, to note, if possible the first manifestations of new cases. Just as soon as a new case is found the patient is at once taken care of in an isolated hospital. Suspects are kept under observation in an isolated camp.

All personal effects, including every bit of clothing worn, is disinfected in enormous double walled cylinders, by means of hot formalin laden steam under pressure. Sterilization is made absolutely complete without any injury to the clothing.

The ship with its cargo is next disinfected with sulphur dioxide gas generated in iron pots or pails placed upon sheets of tin. A little alcohol is poured over the sulphur, ignited, the exits closed down and kept closed for twelve hours. If the cargo contains combustible material as alcohol, oil,

of health: Scarlet fever (including scarletina and scarlet rash), diphtheria (including membranous croup), small-pox, epidemic cerebro-spinal meningitis, anterior poliomyelitis, leprosy, and bubonic plague.

benzine, etc., the sulphur dioxide is generated upon a special boat or float which is run alongside and the fumes conducted into the hold of the ship to be disinfected. The sulphur fumes kill all organisms present, including fleas, rats and mice. In fact sulphuring of ships must be resorted to quite frequently for the sole purpose of killing rats and mice, even though there may have been no disease on board.

### 4. Purification and Sterilization of Water Supplies.

Every city, town, hamlet and home should have an ample supply of pure water for drinking, cooking and cleansing purposes. Impure waters, that is waters which require sterilization in order to render them potable, are always dangerous. It is therefore of prime importance to secure a pure supply of water, sufficiently pure to make the work of sterilization and purification wholly unnecessary; if that is not possible, and it generally is not, under our peculiar communal condition, then said questionable water supply should be thoroughly sterilized and purified, according to the most approved modern methods. We cannot condemn too strongly the generally prevalent methods of emptying the sewage of our cities and towns into rivers and lakes and then again supplying this sewage contaminated water to towns and cities for drinking and cooking purposes. There should be an efficient state board of health coöperating with a Federal department, and there should be efficient and competent sanitary inspectors to look after the water supplies of private homes, of towns and in the country.

The suitability of water for drinking purposes is inversely proportional to the number of bacteria present. Pure spring or well water contains very few bacteria, rarely exceeding 50 per c.c. Sewage contaminated water, which is still used for drinking and cooking purposes, may contain several million bacteria per c.c. It has been proven time and again (statistically) that the mortality rate (due to disease) of cities is practically proportional to the purity of the drinking water supply. It is self evident that water purification should be considered a subject of the utmost importance. It should receive more attention than it does.

The sedimentation and filtration method for removing dirt, sand and other coarser particles from the water supplies of large cities is practised and has been practised for years in many of the European cities. This is satisfactory as far as it goes, but it does not go far enough. The filtering material used (sand, charcoal, etc.) does not remove bacteria and other small organisms, excepting those which are attached to the coarser particles remaining upon the filtering material. Furthermore, unless the filter is frequently changed or sterilized, the filtering material will become the breeding place of germs and thus contaminate the water still more.

Various chemical disinfectants have been tried, but most of them have

proven unsatisfactory for various reasons. The use of high attenuations (1–5,000,000 to 1–50,000) of copper sulphate has been highly recommended, especially by the U. S. Dept. of Agriculture, and has in many instances given excellent results, especially in the destruction of low forms of algæ and protozoa. As a means of destroying bacterial life the method is, however, not a success. Dr. Kraemer and others recommend the use of copper foil or plates immersed in the water as a means of destroying pathogenic and other bacteria, but this method does not appear to have met with any general approval. Kraemer sums up the copper foil treatment of water as follows:

1. The intestinal bacteria, like colon and typhoid, are completely destroyed by placing clean copper foil in the water containing them.

2. The effects of colloidal copper and copper sulphate in the purification of drinking water are in a quantitative sense much like those of filtration, only the organisms are completely destroyed.

3. Pending the introduction of the copper treatment of water on a large scale the householder may avail himself of a method for the purifications of drinking water by the use of strips of copper foil about 3 1/2 inches square to each quart of water, this being allowed to stand over night, or from six to eight hours, at the ordinary temperature, and then the water drawn off or the copper foil removed.

The alum method of purifying water has met with considerable success, but more recently the alum-sodium hypochlorite combination has proven more satisfactory. The alum coagulates and precipitates the organic impurities and the sodium hypochlorite, through its electric dissociation, acts as a germ destroyer. The coagulated and precipitated organic material holding most of the bacteria is then removed by filtration. The amount of chemicals used depends somewhat upon the degree of contamination. With highly contaminated waters it is customary to use 3.3 per cent. of alum as the coagulant, subsequently introducting 1.2 per cent. of the hypochlorite. The water is then filtered, whereupon it is ready for use.

Small quantities of drinking water may be purified as follows: Dissolve a level teaspoonful of powdered chloride of lime in a teacup of water. This solution is diluted with three cupfuls of water, and a teaspoonful of the whole quantity is added to each two-gallon pail of drinking water. This will give 0.4 or 0.5 part of free chlorine to a million parts of water and will, in ten minutes, destroy all typhoid and colon bacilli or other dysentery-producing organisms in the water. Moreover, all traces of chlorine will disappear rapidly.

There are in use a number of methods for dissociating sodium hypochlorite by electricity. Some of them are patented and modifications thereof are in use by city water purification works, giving excellent results. Dr. C. P.

Hoover, assistant chemist of the Columbus Board of Health, has the following to say regarding the process:

"There are two general types of electrolyzers for dissociating sodium chloride. In one the cathodic and anodic products are allowed to recombine in the main body of the electroyte and in the other, known as the diaphragm process, the products are removed separately from the cell as produced.

"For the production of sodium hypochlorite the non-diaphragm process has been considered best because it dispenses with the destructible diaphragms and the loss of energy that all such diaphragms occasion.

"When a direct current of electricity is passed through a solution of sodium chloride, sodium is liberated at one pole and chlorine at the other. The liberated sodium reacts on the water breaking it up into hydrogen and hydroxyl ions to form sodium hydrate. The sodium hydrate in turn combines with the chlorine to form sodium hypochlorite, (Na O Cl) which becomes active in the sterilization of the water."

Pharmacists find considerable demand for distilled water for drinking purposes as well as for use in dispensing. However, some of the leading authorities declare that drinking distilled water is objectionable, because of the disturbance of the osmotic pressure in the cells of the digestive tract. That is, the distilled water acts as a mechanical poison. There is an excessive endosmosis inducing an abnormal distention of the cells, causing physiological disturbances. This action is due to the fact that the mineral salts present in natural drinking water are absent in distilled water.

The pharmacist can prepare cheaply and simply a marketable drinking water which does not have the objectionable qualities above referred to. Instead of distilling the water, filter it, using a Pasteur-Chamberland filter. Whether a large or small filter is used will depend upon the number of customers to be supplied. In all probability a two- or three-tube filter is large enough for the average retail store. "Rapid safety filters" are of no value whatever, and should not be used, as they are in no sense germ-proof. They merely remove the coarse filth. It is true that the Pasteur-Chamberland filters are not absolutely germ-proof, but they remove most of the microbes present, as may be determined bacteriologically by the pharmacist himself. The few germs which may pass through the filter are killed by heating the water to the boiling-point or 30 minutes. Such filtered and heat sterilized water should be sold in large sterile glass or earthenware containers. It is more palatable than distilled water and does not interfere with the physiological action of cells.

### 5. Food Preservatives.

The use of food preservatives is as old as the history of man. Since remotest antiquity man has found it necessary to accumulate a supply of food

during the seasonal periods of plenty in order to tide over the periods of scarcity. The very first observation made was that the accumulated and stored food soon showed a tendency to undergo decomposition. The next observation no doubt was that under certain conditions some organic food kept better than under other conditions, thus, for example, primitive man gradually learned that sun-dried meats did not decompose nearly as quickly as undried meats. No doubt the value of smoking meats was soon ascertained, in all probability purely accidentally, from meats, etc., which had been exposed to the smoke of the camp fire. The preservative value of heat, as in cooking and roasting, was noted. Next, no doubt the preservative properties of certain chemicals used with foods, as ashes from the camp fire, salt, brine, vinegar, wine (alcoholic beverages) and sugar was noted. Thus primitive man made use of the germicidal powers of sunlight, drying, dry heat, moist heat, wood ash, smoke, creosote (in smoking meats), salt solutions, acids (in vinegar) and alcohol, without having any idea as to why these agents retarded or prevented the decomposition of organic food substances.

In modern times the use of food preservatives is based upon the germ theory of decomposition. The time-honored preservatives above referred to have continued in use and many new ones have been added, as benzoic acid, sodium benzoate, boracic acid, borax, salicylic acid, sodium sulphite, sulphurous acid, formalin and many others. A somewhat generalized theoretical assumption is that the chemical preservatives in foods are more or less injurious to health. It cannot be denied that some of the preservatives used are irritating to the kidneys and skin and some perhaps interfere more or less with food digestion and assimilation. It has long been known, for example, that the prolonged consumption of salted meats produces serious skin affections designated as scurvy. The sulphites are irritating to the kidneys; formalin interferes with digestion of foods, etc. However, there can be little doubt that in the comparative sense it is far more conducive to health and longevity to eat preserved foods than foods which are more or less decomposed. We are daily making use of foods which contain small quantities of natural preservatives. Cranberries, for example, contain benzoic acid; formalin and phloroglucin are present in minute quantities in certain plants; a multitudinous variety of salts, acids, sugars, aromatic oils, etc., are present in food plants. Food chemists do not appear to be seriously worried about these natural preserving agents nor about the old-time artificial preservatives as smoke creosote, salt, brine, sugar, and vinegar, and it is reasonable to suppose that careful investigation will disclose new chemical preservatives which are superior to those mentioned. The whole discussion regarding artificially added chemical food preservatives will no doubt simmer down to the following: *What is the smallest amount of the least objec-*

*tionable chemical food preservatives which must be added to certain food substances in order to preserve them until they are to be consumed?* Also the following correlative rule should hold good: *No chemical food preservatives whatsoever should be used as such excepting in cases were modern methods of heat and cold sterilization and preservation fail or are inapplicable.*

The use of sugar and of salt in moderation are, of course, always permissible, since these substances are essentials in many foods. The objection and danger in the use of food preservatives lie in the fact that careless manufacturers are too prone to use them in order to avoid employing harmless, though perhaps less simple, and more expensive means of food preservation. Chemical preservatives make it possible for the unscrupulous to use decomposed and otherwise objectionable food material. Furthermore, there is a strong tendency to use chemical preservatives in excess, in spite of the strictest legal quantitative limitations.

The following is a brief summary of the more common food preservatives and their use.

The physical and mechanical means of food preservation have been referred to, likewise the use of heat, cold, smoke, etc. One of the most satisfactory methods of preserving foods, now employed in all up to date canneries, is a combination of heat sterilization with air exclusion (air pump and by displacement). The food products as meat, corn, beans, asparagus, peas, jams, jellies, preserves, etc., etc., are heated (100° C.) to destroy all germ life, the containers (tins, glass) are also heated and then entirely filled to exclude as much air (oxygen) as possible. Air (oxygen) is necessary for the growth of bacteria, yeasts and moulds, hence a well filled container, with a minimum of oxygen is less likely to show decomposition effects ("swells," "leaks") than containers which are not well filled. It is claimed that wholesome fruit, meat, etc., (free from decomposition), which is well sterilized by steam heat and put up in well sterilized containers requires no chemical preservative whatever. It is, however, customary, in the case of fruits, to add sugar as a preservative and also for the purpose of rendering the article more palatable. The sugar from sugar cane is quite universally used in preference to the sugar from the sugar beet. This is no doubt due to the fact that sugar beet sugar contains slightly more organic impurities and is, hence, under similar methods of use as to quantity, degree of heat sterilization, etc., slightly more likely to undergo decomposition.

Preservation of food substances by drying is coming into use more and more. By this method it is possible to keep, for variable periods of time, a great variety of foods as apples, peaches, pears, bananas, potatoes and many other vegetables, besides bread, meats, eggs, milk and other substances, which were formerly more generally preserved by the canning method. Eggs may also be preserved entire by giving them a coating of tallow, wax,

paraffin or soluble silicate, which exclude the air, or they may be preserved in brine, salt or other so-called harmless chemical preservative.

Herring, cod and other fish are often preserved in a brine of salt or of equal parts of salt and borax or boric acid. Of meats, fish is particularly liable to decomposition and it is declared that certain kinds cannot be preserved in salt alone, that it is necessary to add boric acid, rubbing the preservative well into incisions made along the spinal column where the decomposition develops earliest. Salt is used with meats generally and with butter. Two per cent. of salt in butter is sufficient, though as much as 15 per cent. and more is sometimes added to increase the weight. A combination of salt and saltpeter is added to meat (brine). The saltpeter gives a red tint to meat besides serving as a preservative. Saltpeter is considered more or less injurious to health, when taken with food to the amount of 0.5 of 1 per cent. or more.

Borax and boric acid is often added to milk. 4.4 grains to the pint (0.05 per cent.) keeps milk sweet for a time (10 to 14 hours and longer). Small doses of borax and boric acid (up to 1 gram per day) is considered harmless. Certain preservatives of a proprietary nature as "Preserving Salts," "Preservative," consist of borax and salt in the proportion of three to one.

Formalin (the 40 per cent. commercial solution) added to milk, to the amount of 1–50,000, retards souring for several hours; 1–10,000 prevents souring for twelve hours and longer, and in this amount it does perhaps very little harm, though it is believed, due to its coagulating effects, to interfere with the digestibility of milk, particularly in children. Several marketed milk preservatives have formalin for their principal ingredient ("milk-sweet," "iceline," "freezine").

Sulphurous acid and sulphites are added to vinegar, pickles, catsups, etc., anchovy pastes, canned and dried fruits, etc., to the amounts of 0.2 to 1.15 per cent. The part active as a preservative is the available $SO_2$ which is gradually oxidized into sulphates. These agents are deodorant, as well as preservative, because of the high oxidizing power.

Butchers use sulphite preservatives to dust over sausage meats for the double purpose of giving the meat a red color (due to the O combining with the hemaglobin of the blood) and to destroy possible odors of decomposition. 0.05 per cent. of sulphites is sufficient to check decomposition in fresh meats, though the best results follow the use of 0.5 per cent. 0.2 per cent has germicidal powers when combined with cold. Sometimes aniline color is added to the sausage meat preservatives.

Sodium benzoate is perhaps the most extensively employed preservative and at the same time the least harmful. 0.1 per cent. added to food articles, as meats, fruits, catsups, vinegar, cider, etc., checks decomposition. Generally, however, more than 0.1 per cent. is added, from 0.2 to 0.5 per cent.

The percentage of benzoate preservative is likely to vary because of its volatile nature; canners quite generally add an excess knowing that much of it will be carried off with the vapors escaping during the heating process. As a result it follows that products declared to contain 0.1 per cent. of benzoate may upon chemical examination show the actual amounts to range from a mere trace (0.05 per cent. to 0.5 per cent.).

Next to benzoate, salicylic acid is perhaps the most common food preservative, used much like benzoate, in strengths varying from 0.10 to 2.5 per cent. It is frequently added to beers, cordials, wines and foods (4 to 8 grains to the pint) containing sugars. It is also used as a surgical dressing, but other less irritating wound disinfectants are given the preference.

Crude pyroligneous acid is used as a meat preservative. This acid is obtained by the destructive distillation of wood and contains creosote and other tarry matter and imparts the odor and taste of smoked products. Meats, fish, etc., are immersed in a solution of this acid, dried and sold as smoked. This constitutes the "quick" or "dip" method of smoking meats as compared with the usual slower method of exposing the meats to the smoke of slowly burning wood.

The following are a few of the less commonly employed preservatives: Fluorine compounds are used in strengths of from 0.03 to 0.02 per cent. Alum is sometimes used in pickling vegetables and meats (brine) because of the hardening effects produced. Copper sulphate is much used in pickling cucumbers, peas, string beans and other green vegetables for the purpose of deepening the green color. Sodium and calcium carbonate are sometimes added to cider and wine to check the souring process (by combining with the fruit acids). Formic acid is a powerful preservative. 0.014 to 0.08 per cent. retards fermentation. Saccharin, sucrol and dulcin are sweetening as well as preserving agents. Peroxide of hydrogen is used as a preservative. It is also a deodorant. The use of saccharin in food is no longer permissible in the United States.

### 6. Insecticides and Other Pest Exterminators.

The farmer, fruitgrower and florist have many enemies belonging to the insecta and to other divisions of the animal kingdom, which interfere with the productiveness of crops. The remedies employed against these pests are numerous. We shall mention only a few of the more useful ones, explaining their action very briefly. They may be grouped into powders, gases, sprays and washes.

A. *Powders.*—These may be applied by the "pepper box" method, the material being placed in a box, usually of tin, with perforations, through which the powder sifts on shaking. Or a blowing device may be used,

like the ordinary bellows box for blowing insect powder, or modifications of this simple device. A third method known as the sifting method is much in vogue in the cotton fields. The powder is placed in a porous bag or cloth, fastened to a stick and shaken over the plants to be treated. Only three powders are used to any considerable extent, as follows:

a. *Slaked Lime.*—Dry air slaked lime is reduced to a uniformly fine powder which is then ready for use. It is very efficacious with all slimy animals, as slugs and snails. It is applied to plants when the pests are active, that is, in the early morning or in the evening. Lime is used where paris green is not permissible, as with fruit plants and edible herbs.

b. *Sulphur.*—The flower of sulphur or ground sulphur is a very widely used remedy for fungous pests, as mildew; also for the red spider and thrips. Sulphur is active only in the sunlight, particularly on a hot day.

The flower of sulphur gives better results than the ground sulphur because it "sticks" better. It should be applied evenly and not too thickly. Remember that sulphur dioxide is very injurious to plants, therefore fumigation by burning sulphur is out of the question.

c. *Paris Green and Other Arsenicals.*—These are generally not used in the dry powdered form. When so used they are diluted with flour, dust or other inert powdered material. Must be sparingly applied and evenly distributed, otherwise serious damage may be done to the foliage.

B. *Gases.*—Gases diffuse with great rapidity and when applied within an enclosed space will, in a short time, be uniformly distributed throughout the enclosed space. The rapid diffusion of gases is a great hindrance to their practical utilization in the open as in orchards, fields and gardens. Their use is quite limited.

a. *Carbon Bisulphide.*—This is not used with growing plants though it is applied to stored seeds, and dry plants and grains, for the purpose of killing insects and other destructive animals. It is also used to kill pests which live in the soil, as the grape Phylloxera. For this purpose a machine is used which injects the bisulphide into the soil. To destroy pests in drug plants, seeds and grain, enclose them in a space, place a dish containing the bisulphide on top of the material. The vapor being heavier than the air, gravitates downward and soon fills the entire enclosed area. The amount necessary to do the work will depend upon the nature of the material to be treated and the tightness of the enclosure. Roughly estimated a dram of the carbon bisulphide to five pounds of the material is sufficient. Grainmen usually apply one pound to the ton of grain, if the bin is tight.

Carbon bisulphide is one of the most effective remedies against the gopher and the ground squirrel. Use the remedy after a rain as the soil is then less porous. Pour an ounce over a rag or other porous substance (horse droppings are much used), stuff this into the hole and plug with a ball of dirt.

The bisulphide is also used to kill the yellow-jacket, which is very injurious to fruit, also the root crown borer of the peach, and to disinfect grapevine cuttings, etc., etc.

b. *Hydrocyanic Acid Gas.*—This is about the only gas which is powerful enough to kill insects and yet not injure the foliage. It is used by covering the tree, shrub or bush with a tent cloth or canvas which should be oiled to keep in the vapor. The vessel containing the chemicals is placed underneath Exposure of from thirty to fifty minutes is usually sufficient. About one ounce of potassium cyanide to 150 cubic feet of space is required.

The gas is extremely poisonous and is often destructive to foliage. It is preferably applied at night as it is then less injurious to the foliage.

C. *Sprays and Washes.*—Plant pests are most generally destroyed by spraying agents or washes. A wash is really a more liberal application of the spray, the two being alike as to the results to be attained from their use

For low plants the remedy can be applied by means of a sprinkling can but the better method is to use some form of force pump with spray nozzle. A good spray pump should maintain a uniformly constant as well as adequate pressure, should be simple of construction, with all parts readily replaceable. The nozzle should break up the stream into a fine mist.

It is, of course, desirable to get as much as possible of the spray to remain on leaf or stem and to have it evenly distributed. If put on too abundantly the fine droplets or gobules on the leaf will run together and roll off to the ground. The nozzle must not be held near the plant to be sprayed in order to get the disirable dew-like deposit on the leaf.

For scale insects a thorough moistening is necessary, wetting the bark, the scale and eggs. In order to accomplish this the nozzle must be held close.

The following table by Woodworth will indicate the method of preparing and using the more important spraying solutions:

The well known Bordeaux mixture, so extensively used as a spray and wash is prepared as follows:

| | |
|---|---|
| Water, | 50 gal. |
| Copper sulphate, | 6 lb. |
| Unslaked lime, | 4 lb. |

The adhesive properties can be increased by adding soft soap in quantity equal to that of the copper sulphate. It is also advisable to dilute the mixture for spring spraying. It is the most effective and perhaps the cheapest fungicide that can be used.

Aphides (plant lice) and similar plant parasites may also be destroyed with weak solutions of alum (1.5 to 2 per cent.). Beetles may be killed by sprinkling a mixture of equal parts of red lead, sugar and flour, near their

TABLE OF FORMULÆ FOR WASHES AND SPRAYS.

| Name. | Strength desired. | Ingredients. | Per cent. | For 5 gallons (oil can). | For 40 gallons (barrel). | Directions for mixing. |
|---|---|---|---|---|---|---|
| Lime, salt and sulphur. For peach worm, San Jose scale, etc. | For winter use only.. | Lime..... | 9.00 | 3 lbs. | 24 lbs. | Boil sulphur and on half the lime 1 1/2 hours; mix and add other ingredients and boil ½ hour longer. |
| | | Salt..... | 3.00 | 1 lb. | 8 lbs. | |
| | | Sulphur..... | 4.50 | 1½ lbs. | 12 lbs. | |
| Sulphide of potash. For red spider. | For summer use..... | Potash..... | .80 | 4⅓ oz. | 2 lbs. | Mix these with a very little salt and water and dilute after reaction is complete. |
| | | Sulphur..... | .92 | 5 oz. | 2½ lbs. | |
| Resin soap. For scale and other insects. | Usual strength..... | Resin..... | 2.40 | 1 lb. | 8 lbs. | Boil two hours; dilute with warm water. |
| | | Caustic soda.. | .60 | ¼ lb. | 2 lbs. | |
| | | Fish oil..... | .30 | 2 oz. | 1 pt. | |
| | Extra strong for winter use. | Resin..... | 4.00 | 1½ lbs. | 12 lbs. | |
| | | Caustic soda.. | 1.00 | 6 oz. | 3 lbs. | |
| | | Fish oil..... | .50 | 3 oz. | 1½ lbs. | |
| Kerosene emulsion. For scale and other insects. | Weak for plant lice.. | Soap or milk | .15 | 1 oz. | ½ lb. | Mix hot, with spray pump, 15 minutes; use either sour milk or a soap solution. |
| | | sour. | 2.50 | 1 pt. | 1 gal. | |
| | | Kerosene..... | 5.00 | 2 pts. | 2 gals. | |
| | Usual strength..... | Soap or milk | .25 | 1½ oz. | ¾ lb. | |
| | | sour. | 4.00 | 1½ pts. | 1½ gals. | |
| | | Kerosene..... | 8.00 | 3 pts. | 3 gals. | |
| | Usual strength..... | Soap..... | 2.00 | ¾ lb. | 6 lbs. | Mix 5 minutes. |
| | | Kerosene..... | 8.00 | 3 pts. | 3 gals | |
| Poison. For leaf- and fruit-eating insects. | Usual strength..... | Paris green or other arsenical. | .12 | ¾ oz. | 6 oz. | |

Sufficient water is to be used in each case to make up the amount indicated in the next columns.

hiding places, or a mixture of borax 20 parts and precipitated carbonate of baryta (native witherite will not answer the purpose). A great variety of substances are recommended for the extermination of ants, as borax, camphor, balsam of Peru, spraying with benzine, etc. In lawns and in the open generally (in ant hills) they are most quickly destroyed by means of carbon bisulphide. This kills the ants as well as the larvæ.

The exterminators for pests of all sorts is legion and those especially interested must consult some standard work on formulas such, as the Scientific American Cylcopedia of Formulas (Hopkins).

## CHAPTER XII.

## STERILIZATION AND DISINFECTION IN THE PHARMACY.

It is only within very recent years that sterilization in the pharmacy has received any serious attention. Certain pharmacopeias, notably those of Austria and Beligum, give specific directions regarding the sterilization of certain medicamenta, particularly those intended for hypodermic use. The German, English, Italian, Swiss and other pharmacopeias give directions regarding certain sterilizing processes which may be applied to a few articles. Fischer, Stich, Deniges, Mario, Schoofs and other European investigators have given the subject much attention and have perfected many of the details of procedure.

Some of the non-official methods of sterilization are of very doubtful practicability. Particularly the methods recommended for the sterilization of pharmaceutical solutions by means of the ultra-violet rays and by means of chemical disinfectants. Lesure sums up the use of the ultra-violet rays as follows: "A series of experiments shows that, at present, the ultra-violet rays can scarcely be regarded as a practical means of sterilizing pharmaceutical solutions, such as hypodermic injections. It is not yet possible to sterilize liquids in small closed glass vessels, since the glass absorbs the rays of shortest wave length, which are precisely those of most active sterilizing power. Possibly on a large scale solutions could be sterilized in bulk and then filled, *in vacuo*, into sterilized small receivers. The rays might be useful for substances which are decomposed by treatment in the autoclave. Some substances are, however, so readily decomposed by ultra-violet rays, that their solutions can never be sterilized therewith. Such are solutions of quinine salts, of mercuric iodide, of atoxyl, of eserine, of apomorphine and some glucosides, as for example gentiopicrin. Opaque solutions and suspensions of solids cannot be thus sterilized. The permeability of the different solutions to the rays also varies very greatly. Apart from the question of decomposition, it is found that, in the case of gentiopicrin, completely sterile solutions were not obtained even after an exposure of half an hour; on the other hand ancubin solutions were completely sterilized in thirty seconds." The decomposition changes due to the ultra-violet rays are not clearly understood. The indications are that there are no very marked chemical changes in such substances as cocaine and pilocarpin hydrochloride after three hours' exposure. Arbutin shows a change in a few

minutes. There is so much uncertainty as to the results that the method cannot as yet be recommended for practical use.

The addition of disinfectants to medicines for purposes of sterilization has recently received some attention. The use of formaldehyde, ether, chloroform and alcohol, have been recommended, each having its special use in practice. The general criticisms made regarding the use of the ultra-violet rays also apply here. Currie recommends a formalin method as follows: applicable to infusions of calumba, gentian, quassia and senega "The infusions of calumba and quassia are simply evaporated to one-eighth of their bulk, filtered, and 4 minims of the ordinary 40 per cent. solution of formaldehyde added to each fluid ounce of the concentrated infusion. On dispensing, the requisite amount is put in a shallow basin and brought sharply to the boil, thus dissipating the formaldehyde. The infusion is then diluted to the normal strength with sterilized distilled water. Infusion of gentian is made from gentian root alone, and concentrated. To this is added essence of lemon (1 in 10), and the official tincture of orange in the proportion of 2 fluid drams of the former and 1 fluid ounce of the latter to each pint of the infusion. There is also added 4 minims of 40 per cent. solution of formaldehyde to each fluid ounce of infusion. Infusion of senega is concentrated by evaporation and to prevent precipitation, 5 grains of potassium bicarbonate are added to each fluid ounce of the concentrated solution, and 4 minims of 40 per cent. solution of formaldehyde. In case of both gentian and senega infusion, the formaldehyde is dissipated at the time of dispensing, in the manner already described. The advantages of this process are ease of manipulation, cheapness, and the certainty of the antiseptic condition of the infusion while being kept in stock and until dispensed. The quantity of formaldehyde remaining in the diluted infusion is infinitesimal, and may be ignored for all practical purposes."

It is known that weak solutions of hypodermic and intravenous solutions, unless sterilized, will show numerous bacteria upon standing for a time. One per cent. solutions of pilocarpin, atropin, cocaine, morphine, and fluid-extract of ergot have been found to contain millions of bacteria per c.c. However, 10 per cent. iodoform glycerin, camphorated oil (1 in 10), solutions of apomorphin (0.2 in 20), quinine (1 in 10), antipyrin (5 in 10), cocaine (10 per cent.) are usually quite free from bacteria. In a general way the bacterial content of medicinal solutions decreases directly with the degree of concentration. Pus microbes die at once in ether and in a saturated solution of quinine, whereas they remain active in a 10 per cent. solution of cocaine. A 2 per cent. solution of morphine kills pus microbes in twenty-four hours, while pure glycerin kills them only after an exposure of six to eight days.

A perfectly safe rule for the pharmacist is to consider all medicamenta which he handles and which he may be called upon to dispense, as being

possibly contaminated and to sterilize and disinfect all articles which in his judgment as a qualified pharmacist may require such treatment, in so far as it is practically possible. The retail pharmacist must not place too much confidence in the assertions of comparatively little known manufacturers and wholesale houses, regarding the sterile conditions of the articles which they may supply.

The medicines found in a drug store and dispensed by the pharmacist may be grouped as follows:

### A. Medicines which do not Generally Require Sterilization.

a. For internal administration per mouth. They may be contaminated or may become contaminated on standing for a time. Such medicines should be rejected. Do not attempt to render them usable by sterilization.

b. Mouth washes and gargles.

c. Enemas. Enemas for young children and such enemas as are to be applied to inflamed or otherwise pathologic conditions of the intestinal mucous membrane, should be sterilized.

d. Medicamenta which are to be applied to the intact skin, or to the scalp.

### B. Medicines Which Require Sterilization.

a. Those intended for intravenous and hypodermic use. Not only must these be absolutely sterile but they must be in perfect solution, before using.

b. Those to be applied to cuts, bruises, abrasions, wounds, ulcers, sores, and to the broken skin generally.

c. Those to be applied to inflamed mucous membranes, as enemas, douches, etc.

d. Solutions for the irrigation of the bladder.

e. Eye medicines, as washes and other solutions, intended for direct application to the eye.

### 1. Methods of Sterilization.

The following methods of sterilization are applicable in the pharmacy and should be consistently practised:

A. *Sterilization of Containers.*—The glassware and other containers used in the pharmacy should be cleaned and sterilized as follows:

a. *Bottles and Glassware Generally.*—Wash and rinse in warm water to remove dust, dirt, sand, straw, etc., then wash and rinse in hot water with 2 to 5 per cent. sodic hydrate. Neutralize the sodic hydrate by washing and rinsing in 2 to 5 per cent. hydrochloric acid. Finally wash and rinse in hot sterile water and allow to drain. Wipe dry and plug lightly with

cotton. Place the plugged bottles in a hot-air sterilizer and heat for one hour at 120° C. to 130° C. Keep these cleaned, sterilized, and cotton-plugged bottles in clean container in a dry clean store-room, until wanted for use.

b. *Porcelain and Similar Containers.*—May be cleaned and sterilized like glassware. Plugging with cotton is as a rule inadmissible.

c. *Large Flasks, Jugs, Etc.*—Large containers are as a rule difficult to sterilize and for this very reason are often subject to special neglect. Proceed much as for bottles, observing greater caution as to changes in temperature. Large bottles, carboys and similar containers cannot be sterilized by means of boiling hot water as they are very apt to crack. They may be sterilized by means of carbolic acid (5 per cent.), lysol (1.5 per cent.) or formaldehyde (4 per cent.), then thoroughly rinsed in sterile water, allowed to drain, plugged with cotton, carefully heated in hot-air sterilizer for one hour or more at 115° to 120° C. Cool gradually.

d. *Tin Containers.*—Wash and rinse thoroughly in water; boil for thirty minutes, drain and dry and sterilize in dry-air sterilizer for one hour at 100° C.

B. *Sterilization of Apparatus and Tools.*—It is of the highest importance that mortar and pestle, spatulas, percolators, pill and suppository machines, mixing plates, etc., etc., should be clean and sterile. This means a liberal use of hot water, green or soft soap, and clean towels. The sink, the floor of the dispensing room, the tables, chairs, desks, in fact everything in and about the dispensing room should be scrupulously clean.

C. *Sterilization of Corks and Other Stoppers for Containers.*—It would be energy wasted to clean and sterilize the containers if the stoppers are not also clean and sterile. Sterilize corks by washing in hot 60 to 75 per cent. alcohol, drain and heat in hot-air sterilizer for one hour at 130° C. Keep these corks in sterilized wide-mouthed ground-glass capped bottles. Take out corks as wanted by means of a sterile pair of pincers, not by means of fingers. Other stoppers, as of glass, of wood, of rubber, must also be cleaned and sterilized. Rubber caps, rubber stoppers, and other rubber goods may be sterilized by boiling in water for thirty minutes.

D. *Sterilization of Surgical Supplies.*—a. Bandaging materials, cotton, absorbent gauze, etc., may be sterilized by wrapping in cheese cloth or filter paper, first placing a grain of fuchsin or other aniline dye in the center of the package (wrapped in paper or cloth), and sterilizing in steam for one hour. The dye particle is introduced as a test object to ascertain if the steam has penetrated the entire package. If it has penetrated the entire package it will be indicated by a spreading of the color. Afterward, dry for one hour at 100° C. in the hot-air sterilizer. For this purpose the form of Arnold steam sterilizer shown in Fig. 18 will be found very useful.

b. Sewing materials, such as needles, forceps, catgut, etc., require careful sterilization before using. All metal instruments and appliances, including

silver wire, can be sterilized in 5 per cent. carbolic acid if necessary or they may be boiled for 30 to 50 minutes. Wipe perfectly dry with sterile towels and place in hot-air sterilizer for one hour at 100° C. In order to keep them in sterile condition for immediate use they must be kept wrapped in sterilized cloth or cotton.

c. Catgut requires thorough sterilization as not infrequently spores of disease germs (as anthrax) are present. The so-called cumol (cumene) method of catgut sterilization is quite generally adopted in the hospitals of Germany and of other European countries. Wind the catgut in the usual ring form, dry in hot-air sterilizer for two hours at 70° C., place rings in a vessel (beaker, etc.) with cumol on sand-bath and heat to 155° C. or 165° C. (the boiling-point of cumol), turn off the gas and allow to remain in the hot cumol for one hour. The cumol dish should be covered with a fine mesh wire screen to guard against catching fire. Take the catgut rings out of the cumol by means of sterile pincers and place in benzine for three hours, then allow the benzine to evaporate in sterile Petri dishes.

d. Silver catgut is preferably sterilized in 1 per cent. silver citrate (itrol) or 1 per cent. silver lactate (actol), allowing it to remain for six hours, which destroys even the anthrax spores. Next expose the catgut to light (in sterile dishes) for a day or two, then wind or fasten on glass and preserve in 95 per cent. alcohol with 10 per cent. glycerin. Actol and itrol ionize silver far less actively than silver nitrate, hence their preference.

e. Catheters, drainage tubing and other rubber materials are sterilized by boiling in water with 5 per cent. sodic hydrate. Rubber goods will not stand prolonged and frequent boiling. Do not sterilize metal ware with rubber goods.

e. *Sterilization of Medicines.*—As a rule, medicines which are prepared under aseptic surroundings and conditions do not require sterilization. However, the ideal conditions rarely exist and subsequent sterilizations become desirable and even necessary.

Tooth powders, dusting powders and similar substances may be sterilized at a dry temperature of 70° C., for three to four hours. Salves and pastes are difficult to sterilize. Low temperatures (from 60° C. to 70° C.) for several hours may be employed.

Solutions for subcutaneous injection, for wound irrigation, for bladder irrigation, solutions of boric acid, of tannic acid, aquæ, normal salt solutions and all weaker solutions of chemicals, intended for washes and irrigation in surgery, should be sterilized by boiling for five minutes. Strong solutions of chemicals (as acids, alkalies, etc.) do not require sterilization as they are themselves strongly germicidal.

Alkaloidal and glucosidal solutions, and solutions of alkaloidal salts, tinctures and fluidextracts, should be carefully filtered and sterilized in

sealed containers at a temperature of 60° C., one hour each day for six days. Concentrated alkaloidal solutions may be similarly sterilized. It is not advised to employ a higher temperature for these substances inasmuch as the decomposition changes, if any, which may take place at 100° C. are not clearly understood. To be on the safe side, the lower temperature (60° C.) should be employed.

In the case of solutions or emulsions for hypodermic use, prepared with oil, the oil is first to be treated with alcohol (95 per cent.) to remove the oleic acid. Oily solutions of calomel, yellow oxide of mercury, lecithin, and of camphor are to be prepared with sterile materials, then placed in a boiling-water-bath for ten minutes or in an air-bath at 100° C. An interesting requirement is exacted by the Italian Pharmacopeia as regards the glass of the containers for hypodermic injections: Ten to twelve ampuls or five or six bottles are filled with a clear solution of 1 per cent. mercuric chloride, then sealed. They are then left in an autoclave at 112° C. for half an hour, at the expiration of which time no brownish turbidity should be perceptible.

Some of the points pertaining to the sterilization of alkaloidal, glucosidal and other substances which are quite readily decomposed or altered by light and heat, will be treated under ampuls.

### 2. Preparation of Ampuls.

Ampuls (Lat. ampulla; Fr. ampoule;—a flask) are small glass containers filled with medicinal substances usually in solution. These have come into great prominence within recent years, due to the methods of sterilization now required and practised in well regulated pharmacies. Ampuls are really nothing more than very small flasks, the size being suited to single doses of the medicine, as a rule. They were introduced into France about thirty years ago by Limousin and have now come into general use in France, Italy, Spain, Holland and England. It is only recently that they have come into use in the United States. C. A. Mayo was among the first American writers to publish the first more complete information regarding their origin, manufacture and use. (See Proc. A. Ph. A., vol. 57, 1909.) They are generally adopted by the navies and armies of all civilized countries, because of the advantage which they offer for the preservation, storage and transportation of all manner of medicines, particularly those which require sterilization and which are generally wanted for immediate administration. From the standpoint of the physician they are wonderfully convenient and are great time savers.

Ampuls may have any desired capacity, from 1 c.c. up to 100 c.c., and more, but the more usual capacities are 1 c.c., 2 c.c., 5 c.c., and 10 c.c. They are made of alkali-free glass, white or colored (amber). Those supplied by

French, German and Italian makers are of different forms, as flask-like, bulb-like, spindling, globose, etc.

The following are some of the reasons why ampuls have come into use:

a. Most of the liquid medicamenta and those which are to be dissolved before using have little or no antiseptic power and under the usual conditions

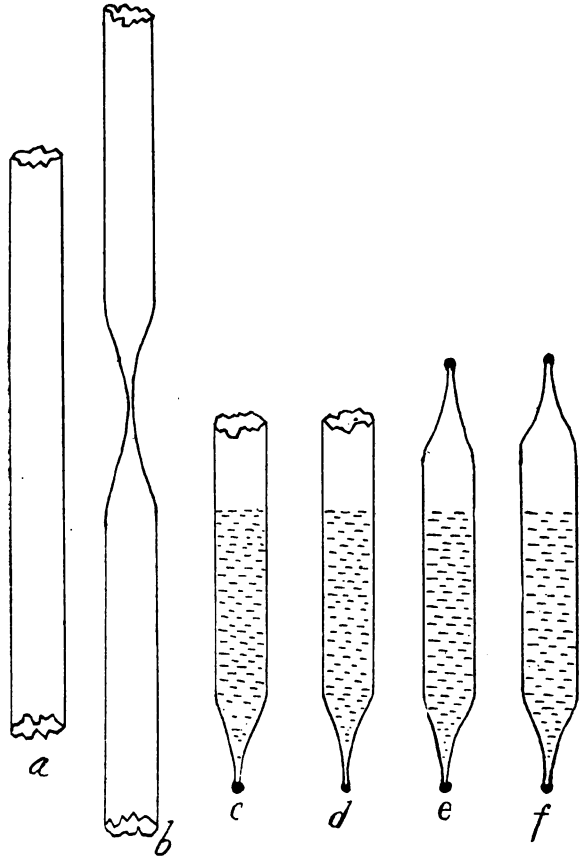


FIG. 75.—Making ampuls. *a*, Piece of glass rod to make two ampuls; *b*, rod *a* heated in the middle and nearly drawn apart; *c*, *d*, two half ampuls filled; *e*, *f*, the completed ampuls.

readily become highly contaminated with different organisms. The use of such contaminated medicines has led to serious infections.

b. The necessity of direct administration of medicinal solutions, by hypodermic, intramuscular and intravenous injection, is due to the desirability of getting prompt therapeutic effects.

c. The direct (hypodermic, intramuscular and intravenous) adminis-

tration of medicamenta is very frequently necessary because administration per mouth is impossible or undesirable.

As a rule the pharmacist will purchase ampuls, ready for immediate use by the physician, from some reliable wholesale manufacturing house. In certain districts and under certain conditions this may not always be possible, in which case the pharmacist must prepare the ampuls. The pharmacist should be prepared to make all ampuls which may be desired by the physicians in his community. The following suggestions can be carried out readily:

A. *Glass Tubing.*—Ampuls can readily be made from ordinary alkali-free glass tubing, selecting rods of a diameter to make ampuls of 1 c.c., 2 c.c., 5 c.c., and 10 c.c. capacity. This tubing can be secured from any chemical or pharmaceutical supply house. Select rods which are quite free from bubbles and of fairly uniform diameter and thickness.

B. *Breaking the Tubing into Suitable Lengths.*—Break the tubing in lengths of from five to six inches, by filing a scratch with a small file and breaking, with the hands protected by gloves to avoid injury by small bits of glass.

C. *Sterilizing and Neutralizing the Glass Tubing.*—Place the lengths of glass rods into water with 5 per cent. of soda and boil for thirty minutes. Neutralize in 5 per cent. hydrochloric acid, rinse thoroughly and again boil in distilled water. Let drain until dry. May be dried in hot-air sterilizer at 140° C.

D. *Making the Half Ampul.*—Take one glass tube and heat the middle part in a bunsen burner with rotation until red hot and soft, and pull apart with a fairly quick strong pull. Break off the thin hairlike ends and hold the tips in the flame to seal them securely. A small bead should form as shown in Fig. 75, c, d, e, f. A little practice with a steady hand is necessary to do this neatly. The half ampuls (one end open, the other sealed as explained) are now laid aside in a sterile box or other container, until ready to be filled. Or the two ends of the ampul can be reduced to a capillary tube as follows. Heat the glass tubing in the blow-pipe flame, beginning at one end, until soft and draw out a short distance with a firm pull. Heat at a point about 1 to 3 inches from the narrowing portion of the glass tube and repeat as before. Repeat this until there are a series of tubes of normal diameter with capillary connections. Breaking these apart with the aid of a file, gives empty ampuls open at the two capillary ends.

E. *Filling the Half Ampuls.*—This can be done by means of a burette, a pipette or a medicine dropper. The burette has many advantages. Many ampuls can be filled from one burette, the exact amounts can easily be measured. The pipette is far less convenient than the burette and is more easily contaminated. A well graduated medicine dropper is very con-

venient. but all things considered the burette is recommended. The points to be kept in mind are.

a. The finished ampul should not be more than three-quarters full. The length (of untapered portion of tube) of a neat looking ampul is about three or four times the diameter of the tubing used.

b. In filling, introduce at least 10 per cent. more than the actual dose required, that is, the 1 c.c. tube should contain 1.10 c.c.; the 5 c.c. tube should contain 5.50 c.c. of the medicinal substance, etc. This is to make sure that the physician may get a full 1 c.c., 5 c.c., etc., dose after allowing for unavoidable loss (portion clinging to inside of ampul, remaining in narrowed ends, etc.).

c. In filling do not allow any of the liquid to come in contact with the upper end (open end) of the tube as that would interfere with sealing.

There are many different methods for filling ampuls which may be classed under three heads; filling by gravity, by pressure, and by vacuum; the latter two being but modifications of the same principle involved. There are on the market (France, Holland, Germany) several devices made expressly for filling and sealing ampuls.

F. *Sealing the Filled Half Ampuls.*—This is done by means of suitable side-flame blow-pipe burner, pinching together and drawing out the soft end of the glass by means of pincers and sealing in same manner as the other end. Do not upend the ampul until it is cool, to avoid cracking the glass.

G. *Sterilizing the Ampuls.*—The hypodermic and other solutions usually put up in ampuls can be divided into three classes or groups according to the degree of heat which may or must be used in sterilizing, namely, those which cannot withstand a temperature above 60° C., those which can be sterilized at 100° C., and those which may be sterilized in an autoclave at 120° C. Inasmuch as the autoclave is rarely usable and also because the ordinary steam temperature (100° C.) will meet all of the requirements of the autoclave, the latter piece of apparatus may be left out of consideration by the practising pharmacist.

To bring about a complete sterilization of the ampuls, the discontinued or fractional method should in all cases be carried out. Place the ampuls in a container (beaker, tumbler, etc.) with water to which enough methyl blue or fuchsin has been added to give it a very marked color and sterilize as follows: If a temperature of 60° C. is to be used, apply this temperature (in incubator with Reichert thermo regulator) for one hour each day for four to eight days. Some manufacturers recommend a period of ten days. If the 100° C. is to be used, apply this temperature (in an ordinary Arnold steam sterilizer) for from 20 to 30 minutes once each day for three days. Should the autoclave be used, an exposure for a period of 20 minutes at 120° C. is sufficient to kill all organisms, including spores.

It is of vital importance in preparing liquids for hypodermic and intravenous injection to have absolutely perfect solutions. There must be no insoluble particles as these might cause serious harm. After the solutions are made they should be forced through a Berkefeld or Pasteur-Chamberland filter. All operations should be done under aseptic conditions, using only chemically pure materials and boiled distilled water. If the contents of the ampuls become cloudy after sterilization or if the inside of the glass tubes show opacities something is wrong and such ampuls should be rejected. Also reject all "leaks," indicated by the aniline color which will appear on the inside of the tube.

The finished ampuls are now ready for use. The physician simply breaks off one end of the ampul, inserts the hypodermic needle (sterilized), upends the ampul and aspirates the contents of the ampul into the syringe by simply drawing down the piston. A second method is to remove the piston from the syringe tube, break off one end of the ampul, insert this end into the open end of the piston tube, break off the other end of the ampul, whereupon the contents will flow into the piston tube; afterward replace the piston rod. In this latter method great care must be observed so as not to get small particles of broken glass into the hypodermic syringe.

Use white glass for making ampuls. Those filled with solutions which are affected by light may be kept in an amber-colored bottle or other container which is impervious to light.

The following substances are commonly put up in ampuls. Many others can be so put up. Each ampul should contain enough material for one dose or for one application, as the case may be. In the columns to the right are given the sterilization temperatures; the preferred or only usable temperatures being given in degrees, the permissible method being indicated by "Yes" and the inadmissible method being indicated by "No." In case of doubt it is always advisable to use the lower temperature (60° C., hourly for from four to eight days).

| Name of Article | Sterilizing Temperatures | | |
|---|---|---|---|
| | Incubator 60° C. | Steam 100° C. | Steam (autoclave) 120° C. |
| Adrenalin | Yes | 100° C. | No |
| Alkaloidal salts generally | 60° C. | Yes? | No |
| Alkaloids generally | 60° C. | Yes? | No |
| Antitoxins | 60° C. | No | No |
| Argyrol | 60° C. | Yes? | No |
| Arsacetin | Yes | 100° C. | No |
| Arsenate of iron | Yes | 100° C. | No |
| Arsenic | Yes | 100° C. | No |

| Name of Article | Sterilizing Temperatures | | |
|---|---|---|---|
| | Incubator 60° C. | Steam 100° C. | Steam (autoclave) 120° C. |
| Atoxyl | 60° C. | No | No |
| Atropin | 60° C. | Yes? | No |
| Bacterins | 60° C. | No | No |
| Cacodylates | 60° C. | No | No |
| Caffeine | Yes | 100° C. | No |
| Caffeine benzoate | 60° C. | No | No |
| Calomel cream | Yes | 100° C. | No |
| Camphorated oil | Yes | 100° C. | Yes |
| Chemicals in solution | Yes | 100° C. | Yes? |
| Cocaine | 60° C. | No | No |
| Duboisine | 60° C. | No | No |
| Ergot | 60° C. | No | No |
| Eserine sulphate | 60° C. | No | No |
| Eucaine | 60° C. | No | No |
| Gelatin | Yes | 100° C. | No |
| Glucosides | 60° C. | No | No |
| Glycerophosphates | 60° C. | No | No |
| Grey oil | Yes | 100° C. | No? |
| Gums | Yes | 100° C. | No |
| Hyoscine | 60° C. | No | No |
| Iron cacodylate | 60° C. | No | No |
| Mercury benzoate | Yes | 100° C. | No |
| Mercury cacodylate | Yes | 100° C. | No |
| Mercury salicylate | Yes | 100° C. | Yes |
| Mercury sozo-iodolate | Yes | 100° C. | Yes |
| Mercurial salts generally | Yes | 100° C. | No |
| Morphine | Yes | 100° C. | No |
| Mucilaginous substances | Yes | 100° C. | No |
| Normal salt solution | Yes | 100° C. | Yes |
| Oils | Yes | 100° C. | Yes |
| Paraffins | Yes | 100° C. | No |
| Quinine | 60° C. | No | No |
| Physostigmine | 60° C. | No | No |
| Salvarsan | Yes | 100° C. | No |
| Scopalamine | 60° C. | No | No |
| Sera | 60° C. | No | No |
| Sodium cacodylate | Yes | 100° C. | No |
| Stovaine | Yes | 100° C. | No |
| Strophantin | Yes | 100° C. | No |
| Strychnine | 60° C. | No | No |
| Toxins | 60° C. | No | No |
| Trypsin | 60° C. | No | No |
| Vaccines | 60° C. | No | No |

Empty ampuls of German and French make can be secured from dealers in glassware and chemical supplies, likewise the appliances for filling and sealing. These ready-made empty and filled, ampuls vary in form as already indicated. Those with a flat bottom and which will remain standing when placed on a flat surface are preferred by some physicians.

The ready-made empty ampuls (still sealed) may be sterilized by boiling for fifteen minutes in a 5 per cent. solution of phenol, rinsing thoroughly in boiling hot sterile water, draining and drying. With the aid of a small sharp file, break off the tips of the ampuls to be filled. Place them in distilled water, bring to a boil, take vessel from the fire for a few moments, pour cold distilled water upon the empty floating ampuls, a partial vacuum is produced in the interior of the ampuls and they quickly fill with water. Now boil for thirty minutes. When water is sufficiently cool take out the ampuls, shake out the water and dry in the hot-air sterilizer at 100° C. They are then ready to be filled, sealed and finally sterilized in the manner already described. An ordinary sterilized hypodermic syringe will be found very satisfactory for filling the ampuls. The suggestions regarding the amount of material to be placed in the ampul, sealing, sterilization, use of the aniline solution, etc., already given, also apply here.

## CHAPTER XIII.

## COMMUNICABLE DISEASES WITH SUGGESTIONS ON PREVENTIVE MEDICINE.

The pharmacist should be prepared to assist the physician and the health authorities in the enforcement of the sanitary rules and regulations. To this end he should be informed as regards the source of the more important contagious and infectious diseases and the causes of epidemics and the means available to prevent or to combat such conditions. This does not mean that the pharmacist must have a full knowledge of the pathology

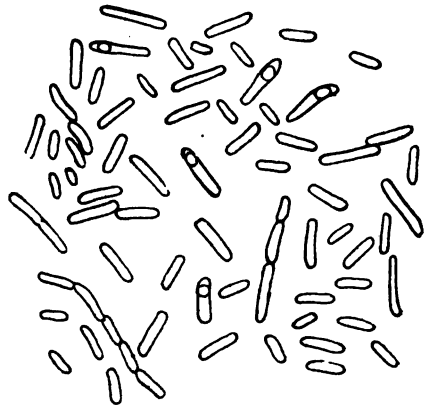

FIG. 76.—*Bacillus botulinus*. This bacillus causes botulism, a form of meat poisoning. There are numerous cases of poisoning resulting from eating infected meats. It should be kept in mind, however, that meat may not be decomposed and may be without bacilli and yet ptomaines may be present. Therefore absence of bacilli and of bad odor does not prove that the meat is wholesome. Meat from animals recently killed, which has been well cared for and which is without bad odor and shows no bacilli, is in all probability wholesome. Ham, canned meats, cold storage meats, etc., may have taken up toxins from contaminated meats, thus being made unfit for consumption even though no bacteria are found.

and therapeutics of disease. He should have at least a general knowledge of the causes of disease in order that he may assist in applying the means for preventing disease. It is not within the province of the pharmacist to cure disease, but he should be a potent factor in preventive medicine.

A contagious disease is one which is readily communicable, from one person or animal to another, either through direct contact or very close proximity. An infectious disease is communicable through a considerable

interval of space. Itch, for example, is contagious, but not in the least infectious, whereas whooping-cough is infectious, but not contagious. Some diseases are both contagious and infectious, as small-pox and diphtheria. Malaria and yellow fever are infectious, but not in the least contagious. However, the distinctions between infectious and contagious are often not very clear. It would be better to discontinue these terms and say that certain diseases are communicable from man to man or from animals to man. When a disease picks its victims rather promiscuously, in a circumscribed area, with none of the usual characteristics of a contagion or infection, we usually apply the term epidemic. For example, cerebro-spinal meningitis and pneumonia may be epidemical. Diphtheria is often

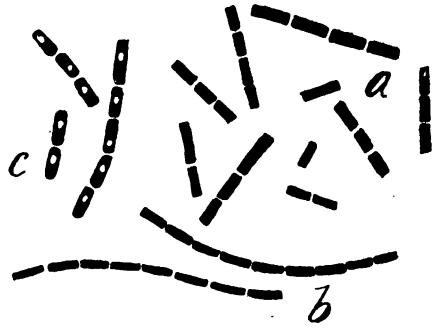


FIG. 77.—*Bacillus anthracis*. This bacillus is spore-forming and causes the cattle disease known as anthrax. This disease is especially common among sheep and cattle and may be transmitted to man, especially those working with the wool, hides and meat of infected animals. The two chief forms of anthrax in man are malignant pustule and woolsorter's disease. The dried spores of this bacillus will live for years and will withstand the boiling temperature for hours. Vaccinating animals against anthrax is commonly practised now. Anthrax is frequently confused with glanders, an equine disease caused by the *Bacillus mallei*. *a*, Non-spore-bearing bacilli; *b*, chains of cells; *c*, spore-bearing bacilli. Cell-walls and plasmic contents are stained, the spores are unstained.

epidemic in a community, and as above stated, it is likewise infectious and contagious. The term epidemic is, however, also applied to any communicable disease which has become general in a given community. A more or less common or spreading disease which is limited to and recurs in a given district or country is said to be endemic in that district or country. Endemics are usually due to climatic conditions which encourage certain microbic and other disease-producing invasions.

The causes of disease are of two kinds, primary or inciting and secondary or predisposing. The primary cause of a disease is that factor or influence which must invariably be active before the disease can possibly develop. For example, the primary cause of diphtheria is the diphtheria bacillus; the predisposing causes are exposure to wet and cold, impoverished condition

of body, etc. No matter how numerous or how active the predisposing causes may be, the disease cannot develop until the primary cause acts. There are numerous abnormal or pathological states or conditions without recognizable primary causes, as gout, rheumatism and the senile changes in the body: and again there are certain diseases which evidently have primary causes, as whooping-cough, small-pox and yellow fever, but in which said primary causes are not yet discovered. The following tabulation outlines the primary and secondary causes of disease:

- Communicable diseases.
  - Primary causes (inciting).
    - Bacteria, as in typhoid and Asiatic cholera.
    - Protozoa, as in malaria.
    - Parasitic higher animals, as tape-worm and itch.
    - Fungi, as in ring-worm and pellagra.
    - Undetermined, as in whooping-cough and small-pox.
  - Secondary causes (predisposing).
    - Heredity.. Race. Family. (Phylogenetic). Individual (ontogenetic).
    - Age..... Infancy. Childhood. Adolescence. Adult. Old age.
    - Sex.
    - Environment.... Climate. Altitude. Seasons. Unsuitable food. Unsuitable clothing. Poisons. Occupation. Injuries.
    - Habits..... Alcoholic. Tobacco. Drugs. Coffee and tea. Gourmandage.

In a general way it may be stated that any cause, factor or influence, which tends to lower the vitality, predisposes to disease. Individuals with a well-balanced physical and mental development are less liable to disease, and when attacked are more apt to recover, than those individuals who have a poor physical development. Undue abstinence is as harmful as over-indulgence. The ascetic is as pathologic as the gouty gourmand.

Irrational diet, drink and food fads, sooner or later leave their pernicious effects upon the system and predispose to certain diseases. Overeating is as objectionable as starvation. Lack of adequate physical exercise has its evil effects as does also over-exertion. Trained or professional athletes

FIG. 78.

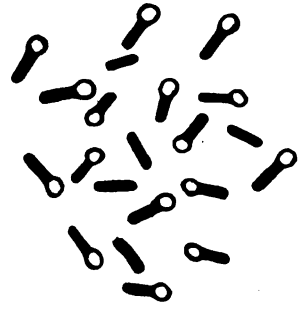

FIG. 79.

FIG. 78.—*Bacillus mallei*, the cause of glanders in horses. This disease can be transmitted to man where it causes symptoms of a suppurative infection of the lymphatic glands. Mallein, which is used in testing horses for glanders, consists of the filtrate (Berkefeld filter) of dead cultures (glycerin bouillon) of the bacillus. A positive malleiu reaction consists in a rise in temperature and local swelling. The dose is 1 c.c.

FIG. 79.—*Bacillus tetani*, an anaerobic spore-bearing bacillus, the cause of tetanus or lockjaw. This bacillus is found in soils and may infect abrasions, cuts and wounds. Treatment with tetanic antitoxin is successful if begun before the symptoms develop. The best time to administer the antitoxin is at the time the injury is received.

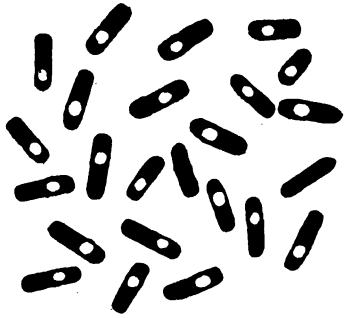

FIG. 80.—A spore-bearing bacillus stained with methyl blue leaving the spores unstained. Fortunately most of the bacilli pathogenic to man do not bear spores.

are not long lived, many are hopelessly afflicted with enlarged and weakened heart and arteries (aneurism). Pernicious habits of all kinds indicate weakness and further develop the weakness, which in turn predisposes to certain diseases and render the individual less resistant to the ravages of disease. A good ancestry and inheritance, good wholesome food, comfort-

able clothing, the right sort of exercise for body and mind, the simple life rather than the strenuous life, avoiding bad habits of all kinds, abundant fresh air, etc., all tend toward longevity. To argue that we should go unclothed is as absurd and unreasonable as to teach that sheep should be shaved. To adhere to a wholly vegetable diet is irrational simply because we are organically adapted to a mixed diet. An excessive meat diet is also very pernicious.

Occupation is a potent factor in predisposing to disease, and in longevity. The following table adapted from a report by Ogle will serve to make this clear. The high mortality rate among street-hawkers is due to several causes chief of which are low-living, exposure to inclement weather, and the greater exposure, in the squalid districts of large cities, to the primary causes of disease. The low mortality rate among clergymen is due to a comparatively simple though comfortable mode of living; while in the case of the farmer and gardener, the out-of-door life is the favorable influence. The list represents ages ranging from twenty-five to sixty years, therefore adults.

| Occupation | Comparative Mortality |
|---|---|
| Clergymen, priests and ministers | 100 |
| Gardeners | 108 |
| Farmers | 114 |
| Carpenters | 147 |
| Lawyers | 152 |
| Coal miners | 160 |
| Bakers | 172 |
| Builders, masons, bricklayers | 174 |
| Blacksmiths | 175 |
| Commercial clerks | 179 |
| Tailors | 189 |
| Cotton manufacturers | 196 |
| Medical men | 202 |
| Stone, slate quarries | 202 |
| Book-binders | 210 |
| Butchers | 211 |
| Glass workers | 214 |
| Plumbers, painters, glaziers | 216 |
| Cutler, scissors makers | 229 |
| Brewers | 245 |
| Innkeeper, liquor dealers | 274 |
| File makers | 300 |
| Earthenware workers | 314 |
| Street hawkers | 338 |
| Inn, hotel service | 396 |

The following are the more important communicable diseases with suggestions on prevention. The information is given for the sole purpose

to better qualify the pharmacist to coöperate with the health officers in safeguarding the public health.

**A. Tuberculosis.**—Commonly known as consumption and the "white plague." A universal disease, essentially infectious, especially peculiar to crowded habitations and to lack of pure fresh air. The primary cause is the *Bacillus tuberculosis* (bacillus of Koch), a non-spore-bearing microbe, which is somewhat more resisting to disinfectants and other destructive agencies than most other pathogenic bacteria. The chief predisposing causes are living in crowded habitations; inherited low vitality, especially weak lungs; and exposure to inclement weather. The disease may be general (general tubercular infection) or it may be localized in any one or in several organs or tissues. Commonly localized in the lungs (phthisis, consumption) and in lymph glands. Lupus and many so-called scrofulous conditions are tuberculosis of the skin; the disease often attacks bones and

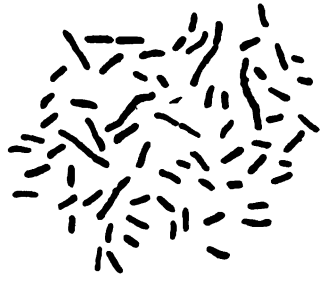

FIG. 81.—*Bacillus tuberculosis.* Although this organism does not form spores it is quite resistant to the action of germicides. The bacillus causing the bovine type of tuberculosis differs slightly in several characteristics from the bacillus of human tuberculosis.

joints (hip-joint disease of children). It attacks young and old and may occur in all walks of life. The disease enters *via* the air passages and per mouth with food and drink, or through cuts, bruises, wounds and abrasions. It is contracted by inhalation through close association with consumptives, and the bovine form or type of tuberculosis is acquired from the milk of tubercular cows. Bovine tuberculosis is especially liable to affect the lymph glands and the joints.

The disease sometimes runs a quick course (quick consumption), but more generally it makes an insidious start and runs a chronic course. Many people have limited local infections which are only discovered at an autopsy. There are many spontaneous recoveries from tuberculosis. Since it is very important to begin early treatment, the physician resorts to several tests for the purpose of determining the possible existence of masked or incipient forms of the disease. These tests are as follows and all depend

upon the reactions produced by tuberculins when introduced into the system:

a. *The Calmette or Ophthalmo Test.*—Old tuberculin, precipitated by alcohol is used. The precipitate is dried and made into a 1 per cent. solution in sterilized distilled water or sterile physiologic salt solution. This substance is put up in sterile capillary pipettes, ready for use. A drop of the solution is placed in one eye, using the other eye as a control. Any abnormality in the eye is regarded as a contraindication. If tuberculosis exists in the system it is indicated by an inflammation in the eye tested. Also known as the Wolff-Eisner test or reaction. It may be necessary to repeat the test several times before satisfactory results are obtained.

b. *The von Pirquet or Cutaneous Test.*—A 25-per cent. solution of tuberculin (O. T.) is applied to the skin with scarification, as in vaccination. The skin is first cleansed with alcohol and control scarifications are made near the test area. This test is also known as the "skin reaction." It is not very reliable. The inflammatory reaction may be simulated by other substances in persons that are known to be entirely free from tuberculosis.

c. *The Moro, Percutaneous or Ointment Test.*—Fifty per cent. tuberculin (O. T.) in lanolin is rubbed into the skin, without scarification. The preparation is put up in collapsible tubes, one tube containing enough material for several tests. If tuberculosis exists, small reddened vesicles appear at the point of inunction, usually on the second day.

d. *The Thermal Test.*—A solution of tuberculin (O. T.), put up in 8 c.c. bottles, representing one milligram per c.c. (1–1000) is injected hypodermically. If tuberculosis is present there is a rise in temperature, usually within ten to twenty-four hours after injection.

e. *The Detre Differential Test.*—This test is intended to differentiate between tuberculosis of human origin and that of bovine origin. Three tuberculins are required. Tuberculin O. T., tuberculin B. F., made from tubercle bacilli of human origin and tuberculin B. F., made from tubercle bacilli of bovine origin. Three small skin areas are scarified. Into one tuberculin O. T. is rubbed, into the second humanized tuberculin, and into the third bovinized tuberculin. The resulting reactions indicate whether tuberculosis is of human or of bovine origin.

We cannot go into the details of the reactions. They are not always reliable, neither the positive nor the negative reactions. In the advanced stages of tuberculosis and in moribund cases, the reaction is usually negative. Indeed, in such cases the test is unnecessary as the existence of the disease is evident without special tests.

Tuberculosis is not as infectious as is generally supposed. Those who are in good condition physically may live for years with those afflicted with the disease without becoming infected. Yet, tubercular patients should be

isolated from well people as much as possible. The sputum is the principal source of infection, also other secretions; and the breath as in sneezing, laughing and coughing. Plenty of fresh pure dry air should be supplied to patients, large airy sleeping rooms and easily digested wholesome food is essential. Consumptives should not marry, should not kiss healthy individuals, especially children. Expectorated material should be disinfected at once. Treatment should be begun early. The propaganda favoring well constructed, well ventilated, comfortably warmed homes and less close segregation in cities and a general improvement in sanitation will do much toward eradicating tuberculosis. Tenement houses and large or small crowded houses of all kinds should not be tolerated for moral as well as for sanitary reasons. Above all, see to it that the milk used is free from tubercular infection.

**B. Typhoid Fever.**—This is a filth disease. If the environment were made clean and sanitary, typhoid fever could not exist. The primary cause is the non-sporulating *Bacillus typhosis* which is found in filthy water, in milk and in food materials. Slops, sewage, wash water, etc., poured on the soil may seep into the well water and finally enter the system in drinking. The bacillus develops readily in the intestinal tract where the reaction is alkaline. It is quite susceptible to the action of weak acids and is easily killed by boiling and by disinfectants. Typhoid is a widely disseminated dangerous infectious as well as contagious disease. In large cities the mortality rate from this disease is directly proportional to the filthiness of the drinking-water supply. In country districts epidemics are very frequently due to contaminated well-water (contaminated from kitchen refuse, barns, cow-sheds, etc.). Epidemics often follow in the wake of the dairyman, who supplies cow's milk in cans washed with or which contain milk, adulterated with polluted water. Typhoid fever is carried in vegetables from truck gardens where human and other excrement are used for fertilizing purposes. The Chinese truck gardeners are particularly culpable in this regard. Again, the vegetables are irrigated with stagnant and sewage-polluted water. House flies are carriers of typhoid.

The mortality rate in typhoid is high and the disease runs its course in about five weeks. There are some mild cases, the so-called walking or ambulatory cases. All of the excreta from the patient should be disinfected, using corrosive sublimate solution (1–1000), copperas solution (10 to 20 per cent.), blue vitriol solution (5 to 15 per cent.), milk of lime (for stools), etc. All bed linen, clothing, etc., used by the patient should be disinfected in 5-per cent. carbolic acid before washing. Everything used by the patient should be sterilized, disinfected and kept away from the rest of the family. Those who nurse typhoid patients must be extremely careful not to carry the infection to others. Pillows, mattresses and other large articles used by the patient should be steam sterilized, or if that cannot be

done conveniently, they should be destroyed by burning. In simple words, everything about the patient must be scrupulously sterilized in order to avoid spreading the infection.

A national department of health should see to it that the water supply of large cities is free from sewage contamination. Our streams, lakes and reservoirs supplying drinking water require careful guarding against typhoid infection.

There should be a compulsory regulation regarding the position and depth of wells in farm yards and as regards the position of the well relative to barns, cow sheds, privy vaults, etc. Typhoid fever will continue its ravages as long as filth contamination of water supplies and food supplies is permitted.

Fig. 82.—*Bacillus pneumoniæ* of Friedlander, also known as *Bacillus mucosus*. This organism is non-sporogeneous and is easily killed.

The Gruber-Widal test for typhoid is an agglutination phenomenon. The agglutinating power of the blood of a typhoid patient is usually noticeable as early as the fifth day of the disease. Preventive inoculation with typhoid bacterin has been used with considerable success, particularly in the British and German armies, and is now quite extensively used in general practice. Chantamesse and Wright use agar or broth cultures of the typhoid bacillus, killed by heat. It is declared by some physicians that the bacterin administered early in the disease, checks it, and occasionally effects a prompt cure.

**C. Pneumonia.**—Pneumonia, with its modifications, as broncho-pneumonia, capillary bronchitis, pleuro-pneumonia, pneumonic pericarditis, etc., is extremely common. The primary cause is the non-sporogenous *Diplococcus pneumoniæ* (*Micrococcus lanceolatus*). The important predisposing causes are exposure to wet and cold, weak lungs, infancy, old age, general debility and alcoholism. The disease is not very infectious, in fact is not generally so considered. It is generally limited to the respiratory tract and the contiguous tissues, as the pericardium and the pleuræ. Among infants and young children and those well past middle life, the disease shows a high mortality rate. In youth and early middle life recovery is the rule, provided the physical inheritance and development is good. The mortality rate among those addicted to the use of alcoholic drinks, and those affected with "tobacco heart," is very high.

One attack of pneumonia is supposed to increase the resisting power to subsequent attacks but such acquired immunity is not by any means permanent. The anti-pneumococcic serum is used with some apparent success

though the results are far from satisfying to the majority of those who have tried it. Dr. Shafer has recently recommended a mixed bacterin (composed of disease exudate and pure pneumonic bacterin) which has been used with some success.

It is important to guard against exposure to wet and cold, particularly when the vitality of the body is lowered, as through lack of sleep, lack of food, over-exertion, etc. The sputa of patients should be disinfected at once. Well persons having good resisting power may carry the germs and convey the disease to those who have a lower vitality. The room occupied by the patient should be thoroughly fumigated as soon as possible.

**D. Small-pox.**—Also known as variola and pest. This is a well-known disease which has occurred epidemically from time to time throughout all ages and in all lands. It is most highly infectious and contagious. In spite of all investigations, the primary cause has not yet been discovered. The contagion is carried by the breath of the patient, is wafted from the skin eruptions, is carried in clothing and by everything used or touched by the patient. The contagion may lie dormant in clothing for months.

All excreta from the patient should be disinfected with bichloride of mercury (1–1000) or a 5 per cent. solution of carbolic acid or other convenient disinfecting agents as lime, formalin, etc. Bedding, mattresses and other material used in the sick-room should be burned as soon as the patient does not need them any longer.

As the result of the general practice of vaccination (with the modified cow virus) small-pox is no longer the dread disease that it once was. In Germany, where vaccination is carried out with greatest strictness, the mortality rate from small-pox is about 0.1 per cent. In the United States where vaccination is laxly enforced, the mortality rate is about 3 per cent. Since vaccination is almost an absolute safeguard, there is no need of fearing this disease, even when brought in direct contact with it. One vaccination does not establish life immunity, as is popularly believed. The rule is to vaccinate in infancy, again about the time of adolescence and again in early adult life. This will usually insure immunity for life. However, vaccination should be carried out after every exposure or whenever small-pox exists in the vicinity, no matter how many good "take" scars there may be. Nurses and physicians in pest hospitals are vaccinated once a year, or oftener, to insure immunity. In the navy it is customary to vaccinate every man every time a port is entered where small-pox is suspected. Small-pox is a quarantinable disease.

There is absolutely no danger or ill effect from vaccination, in spite of the popular newspaper and popular verbal reports to the contrary. In perhaps one case in a million, tetanus or severe septicemia may be traceable to the use of an impure virus. Septic infection of the scarified area may take

place, due to carelessness on the part of the patient, and not due to the virus used, but even this is an extremely rare occurrence. Since the incubation period of small-pox is about twelve days and that of vaccinia (cow-pox) is only five or six days, it is evident that the vaccination will establish immunity even in those who were actually exposed, provided vaccination is done within a few days aften exposure.

Primitive (savage) races are very susceptible to small-pox, with a very high mortality rate. This is in part due to the total ignorance of sanitary measures, resulting in the more ready spread of the contagion. Entire savage tribes have been exterminated by this disease. Negroes are far more susceptible than Caucasians. Indians have spread the infection in blankets after having been exposed.

**E. Malaria.**—This familiar disease, commonly known as ague, the shakes, chills and fever, and intermittent fever, prevails in many areas in the United States and is limited to swampy wet countries. It gradually disappears with the tilling and the draining of soil which remove the breeding places of the only carriers of the disease, namely the mosquitos (Anopheles). The primary causes is the *Plasmodium malariæ* (*Hæmatozoa malariæ*) which is introduced into the circulation by the sting of the mosquito.

The prophylactic measures consist in the destruction of the mosquitos in rooms. To this end burn two pounds of Pyrethrum to every thousand cubic feet of space. Sulphur one pound per thousand cubic feet may be used though it offers no advantage over the Pyrethrum and has the disadvantage of corroding metal and fading colored fabrics. Also destroy the breeding places of the mosquito and keep mosquitos out of houses by means of screens and netting. Protect the person against mosquito stings when travelling in countries known to be infested by the Anopheles group of mosquito. Also take quinine as a prophylactic (3 to 5 grains twice daily), and as a cure. Quinine is, however, more satisfactory as a preventive than as a cure. The Plasmodium is known to be very susceptible to the action of quinine.

**F. Diphtheria.**—This dread disease is both infectious and contagious. The primary cause is the *Bacillus diphtheriæ* also known as the Klebs-Loeffler bacillus. The chief predisposing causes are exposure to wet and cold. The disease may be localized in the larynx (membranous croup), in the pharynx, in the nares, on any of the mucous membranes, and in cuts and wounds. Animals such as cats may carry the infection. It is also stated that the bacillus is apt to occur in certain soils and in stable manure. The sick must be isolated and all discharges from nose, mouth and throat as well as the bed linen, etc., must be sterilized and disinfected. Upon recovery the sick-room must be thoroughly fumigated by means of formaldehyde.

Bedding, mattress and pillows should be burned. The anti-diphtheric serum should be used early and in large doses. The best authorities look upon this remedy as a specific, always effecting a cure, provided it is given in time and given in adequate doses. All those who have been exposed should receive a prophylactic dose of the remedy (about 500 units). The other remedial agents as gargles, sprays, etc., should not be neglected. The diphtheria toxin acts on the heart and all patients should be warned against any sudden or severe exertion until complete recovery is assured by the attending physician as death has resulted from a single undue action, as jumping or suddenly rising from bed.

**G. Cancer.**—The primary cause, the secondary cause and the treatment of cancer are all in the dark as yet. We know that this disease rarely develops earlier than middle life. It usually runs a comparatively short course (several months to two years), producing some rather marked symptoms (the cancerous cachexia), with constant pain, and a very characteristic waxy pallor of the skin. It is to be hoped that the primary cause and the cure will be discovered in a short time. There are some indications that a tendency to cancer is inherited and that the primary cause is an organism resembling the protozoa group. There is a popular belief that eating raw tomatoes causes cancer, and it may be that the plasmodium of cancer resides in some vegetable. Cancer may attack any tissue or organ, although the internal viscera, as liver and stomach, are more commonly affected. Cancer should be treated as a contagious disease though the proof of its contagious nature is not conclusive.

All advertised cancer cures are fakes. There is no known cure for cancer, unless the Gilman cancer vaccine proves to be one. Surgical removal of cancerous growths has been the means of prolonging life, but the trouble is very apt to recur. Many cases are inoperable.

**H. Plague.**—This disease, which is also known as black plague, the pest bubonic plague, black death, etc., is essentially a filth disease. The primary cause is the non-sporogenous *Bacillus pestis*. The plague has occurred epidemically from time to time throughout all ages. It is most virulent and most prevalent (endemic) in the crowded cities of the warmer countries (Oriental cities), where the sanitary conditions are often very bad. The disease is highly contagious and infectious and is communicable not only to man but also to rats, mice, dogs, squirrels and cattle. Rats, and the fleas upon them, are the principal carriers of the disease, although other animals as ants and flies may also act as carriers.

There are several forms of the plague of which the pneumonic is the most dangerous and most infectious because the bacilli are spread by coughing and sneezing.

In this disease thorough disinfection is of the greatest importance. The

entire body of the patient should be washed with a disinfecting solution (1–1200 bichloride of mercury). Disinfect everything used about the patient. After death or recovery everything used by the patient should be destroyed by burning.

Rats (bearing the infected fleas) are the principal carriers of this disease, and the experience in San Francisco (1906–1909) has demonstrated that plague disappears as soon as the plague infested rats disappear. Destroy rats and mice and see to it that the home is free from fleas. Plague is a quarantinable disease and the federal authorities are constantly on the look-out to prevent the importation of this disease. The Oriental ports are the chief sources of infection.

FIG. 83.

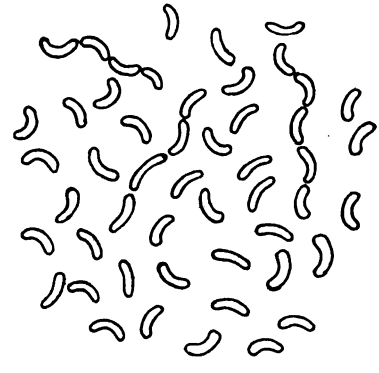

FIG. 84.

FIG. 83.—*Bacillus pestis*. Does not form spores and is very easily killed. The ends stain more heavily than the middle. Involution forms may accur. Sometimes the cells become encapsuled as shown in the figure.

FIG. 84.—*Bacillus choleræ* also known as *Spirillum choleræ*, the cause of Asiatic cholera.

Yersin's anti-plague serum and Haffkine's bacterin have been used with considerable success as prophylactics and also with some success as cures.

**I. Asiatic Cholera.**—This is another filth disease essentially of Oriental origin, particularly prevalent in the crowded unsanitary cities of India and Asia. It is a quarantinable disease. The primary cause is the non-sporogenous *Bacillus choleræ* (*Spirillum choleræ*) also known as the comma bacillus of Koch. The principal sources of the infection are polluted water and food, particularly the former. In fact the sources of infection and modes of entry into the digestive tract are not unlike those of typhoid. Cholera is highly infectious and usually occurs epidemically, often spreading over wide areas. Human excrement carries the infection and when this material is used as fertilizer, which is done in China and other Oriental countries, it becomes the means of initiating and continuing the spread of the disease. The importing by the Chinese of human excrement and animal dung for

medicinal purpose should be prohibited as it may be the means of starting an epidemic of cholera in the United States.

Fortunately the cholera bacillus is easily killed by heat, disinfectants, and by drying. The temperature of boiling water kills it in five minutes. In water it may retain its vitality for a long time. Furthermore, it is not a strict (obligative) parasite and may multiply outside of the body under favorable conditions. Flies carry the infection from cholera stools to articles of food.

Haffkine's attenuated cholera bacterin has been employed successfully as a prophylactic. The method of use consists first in the hypodermic injection of a weak virus, that is, cultures attenuated by long cultivation at a high temperature (39° C.), and following this later, in five days, with a virulent culture. More recently Kolle has used cultures killed by heating for one hour at 58° C., which has given good results in numerous tests made during a cholera epidemic in Japan. Pfeiffer and others have experimented extensively with cholera-immune serum and have demonstrated that this has marked lytic properties. The cholera bacilli when placed into the serum first lose motility, then swell up into coccus-like forms and finally dissolve. This property is said to be due to two substances, one found in normal serum and the other found in immune serum. Neither substance alone can destroy the cholera bacilli but the two acting together are strongly bacteriolytic. The immunity produced by the Haffkine and Kolle bacterins is temporary only.

**J. Yellow Fever.**—This highly infectious, but in no wise contagious, disease is peculiar to tropical and subtropical countries. The primary cause is as yet unknown but it is supposed to be a protozoan. The sole carrier of the infection is a mosquito, *Stegomyia calopus*. The disease has been highly epidemical in the southern states but since the discovery of the part played by the mosquito the mortality rate has been lowered to a marked degree. In fact the disease is now under complete control. No *Stegomyia* mosquitos, no yellow fever.

It had been observed for a long time that a frost checked the disease at once, which as is now known, was due to the fact that the frost killed the carriers of the infection. In a general way the statements made under malaria prophylaxis also apply here. Caucasians, especially those not acclimated in the yellow-fever countries, are very susceptible to the disease; Negroes and Latin races are far less susceptible.

**K. Pellagra.**—Pellagra is a disease which has created great havoc in Italy and other Eastern countries, and which first appeared in the United States about 1907. It spread very rapidly and up to 1911 numerous cases have been reported from the Southeastern United States and from Illinois, with a few scattering cases from Kansas, Virginia, Pennsylvania, New York, Massachusetts, California and other states. The disease is said to

be caused by eating mouldy corn (*Zea mays*) or foods prepared from such corn. Ceni and others declare that the primary cause is a species of Aspergillus (*A. flavescens* and perhaps also *A. fumigatus*). It is also believed that the ordinary household mould (*Penicillium glaucum*) is a primary cause. The mortality rate is very high, and the disease is said to be terrible in its effects. It first manifests itself as an eruption of the skin, usually appearing in the early spring, February or March, after some variable prodromal symptoms. The skin becomes darkened and blotchy. Eczematous eruptions next appear, with desquamation. Gradually, as the older eruptions heal, while new ones form, the skin becomes rough, from which the name, *pell' agra* —rough skin—is derived. The symptoms increase from year to year. The nervous manifestations are varied and are accompanied by great suffering.

Pellagra is not contagious or infectious, though the tendency is transmitted from one generation to another. Children of pellagrins are often born with asymmetrical heads and various other deformities. They may be idiotic or stupid and defective generally.

Acute pellagra runs a rapid course, but more generally it is chronic, the suffering continuing for years in an ever increasing ratio. The sufferers simply degenerate from year to year and die a slow but terrible death.

Lombrosa, Ceni and others recognized the fact that pellagrins are mostly of the poorer class, whose principal diet is polenta, a mush made from corn meal. This mush is usually prepared in large potfuls, sufficient for a week's eating, and set away, exposed to dust, dirt, flies, etc., so that these ignorant peasants often eat polenta which is more or less mouldy and otherwise spoiled. Efforts were at once made to correct these conditions, but proved only partially successful as far as checking the ravages of the disease was concerned.

**L. Syphilis.**—This is a filth disease of which the primary cause is the *Spirochæta pallida*, though several other organisms are generally found present, which have also been designated as being causative of the disease. This is believed to be the most widely disseminated disease of civilization.

It is essentially chronic in its course, the effects being apparent even in the third and fourth generations. Primitive races are said to have been free from this disease until the advent of civilization, yet the disease is of great antiquity having been widespread in ancient Rome and Greece. It is very infectious *via* abrasions, cuts and all breaks in the continuity of the skin and mucous membranes. The infection is carried by all manner of exposed objects, as clothing, dentists' instruments, pipes, dishes, drinking vessels, etc., in fact anything and everything which may have been in contact with a syphilitic. The primary lesions of the patients are very infectious.

The disease is readily preventable. All that is necessary is to keep away from the carriers of the infection. Syphilitics should be isolated until cured. The disease is very readily kept under control by the proper remedial agents, but persistency in the use of medicines is necessary to effect a cure. Ehrlich's 606 (Salvarsan), a new remedy is considered in the nature of a specific, given in hypodermic, intramuscular or intravenous injections.

**M. Gonorrhea.**—This is also a filth disease. The primary cause is the non-sporogenous *Micrococcus* (*Diplococcus*) *gonorrheæ*. It is not infectious but exceedingly contagious to mucous membranes. As *Ophthalmia neonatorum* (opththalmia of the new-born) it is a very fruitful cause of blindness. The

FIG. 85.—Gonococcus and pus cells from the urethral discharges of acute gonorrhea The organism is readily demonstrated by the usual staining methods, using methylene blue or Gram's method. The Gonococcus is cultured with some difficulty (use blood serum-agar in incubator at 37° C.). There are several other cocci resembling the Gonococcus in form, but these differ in that they can be cultured in ordinary media at the room temperature. (*Williams.*)

suppurative discharges from patients are highly contagious. The contagion is carried by patients and by the articles touched or handled by them. The disease is difficult to eradicate from the system. It is not so frequently localized in urethra and vagina as is generally supposed, but it may travel to the bladder, kidneys, joints, etc., and it may be general upon nearly all mucous membranes of the body. It is very apt to become chronic, giving rise to very serious after effects. Syphilis and gonorrhea have the following in common.

1. Both are highly contagious by direct contact, but particularly so to mucous membranes. They are in no sense infectious and are epidemic or general only in proportion to the number of contact inoculations. The

chief carriers and disseminators of the contagions are the women in public houses and the male frequenters of such houses. Lack of personal cleanliness is a very fruitful source of spreading the infection.

2. The innocent (infants, children and adults) are occasionally infected through contact with those afflicted with the diseases, as in shaking hands, kissing, contact with clothing and other articles used by those already infected. Physicians, dentists, and nurses may become accidentally infected. Physicians and dentists may inoculate patients accidentally, through the use of improperly disinfected instruments; this is, however, quite rare. Contaminated drinking vessels, spoons, forks, etc., may convey the infection.

3. In both diseases the primary causes are readily destroyed by the use of disinfectants. With absolute cleanliness the diseases could not exist. In brief, the two diseases could not exist if moral and physical cleanliness prevailed.

4. Both diseases are difficult to cure as already stated. Both are and do become general or systemic in character, and are not local as is generally supposed. Those suffering from these diseases should be isolated and should never be allowed to come in close contact with the innocent.

5. Physicians, pharmacists and nurses should act as public agents in giving information regarding the transmissibility of, and the difficulty of curing syphilis and gonorrhea and pointing to clandestine prostitution as the most active source of the contagion. It should be made a criminal offense for a syphilitic to convey the contagion to an innocent person. In the army and navy the men receive careful instruction as to preventive measures. This was found necessary as the prevalence of these diseases incapacitated a large percentage of the men from active duty.

In the treatment and cure of syphilis mercurial and arsenical preparations and the iodides play a very important part. In the treatment of gonorrhea, disinfectants, especially silver nitrate and protargol, play a very important part. The antigonorrheic bacterin has been used with some success as a prophylactic and as a cure in chronic cases. Only competent physicians can treat these diseases properly. All advertised and patented "quick cure" remedies are fakes.

Recently Ehrlich and Hata have discovered what appears to be a specific in the treatment of syphilis, namely, intramuscular and intravenous injections of dioxydia-amidoarsenobenzol (Salvarsan, or "No. 606"). The tests thus far made have yielded astonishing results. Many of the most severe forms of the disease have been promptly cured by a single dose of this remedy.

The Wassermann or Wassermann-Noguchi test for syphilis is now generally applied to determine whether or not the Spirochæta is in the system. The reaction is due to certain bodies in the blood serum of syphilitic persons

that display a marked affinity for lipoids and in particular, lecithin. Many workers now use, as antigen, an emulsion of lecithin or guinea-pig heart, in place of the watery emulsion of the liver obtained from a syphilitic fetus as described by the originators of the reaction; the advantages being that lecithin and guinea-pig's heart are always on hand and alcoholic extracts are more stable than watery extracts.

The following is an outline of the method of procedure as given by George Gillman of San Francisco.

1. Antigen (a) (original Wassermann); the liver of a syphilitic fetus is cut into very small pieces and an emulsion made of it by shaking with normal salt solution (0.85 per cent.) in the proportion of one (1) part of the liver to five (5) parts of the salt solution. After the shaking is completed, the supernatant liquid is removed and clarified by centrifugalization, after which the clear liquid is pipetted off, one-half of 1 per cent. of phenol added and stored on ice until wanted for use.

(b) If lecithin is to be used as the antigen, it is prepared as follows: Make up a solution of pure lecithin in alcohol; of this alcoholic solution, a quantity equal to 0.1 gm. of lecithin, is added to 100 c.c. of normal salt solution. This is also stored on ice.

(c) Guinea-pig heart extract is prepared as follows: The heart is rubbed up very fine in a mortar (containing ground glass) with absolute alcohol in the proportion of one (1) gram of the heart to 25 c.c. of absolute alcohol. It is then heated to 60° C. for an hour, filtered through filter-paper and kept in the refrigerator ready for use.

As the strength of the antigen will vary in different preparations, it must be standardized before being used. It should be of such strength that the quantity used will not hemolyze 1.0 c.c. of a 5 per cent. suspension of washed lamb's blood-corpuscles in the presence of 0.2 c.c. of a known positive serum, 0.1 c.c. of complement, and 2 minimal units of the hemolytic serum. The unit is determined as follows: A series of test-tubes are prepared each containing the same quantities of the reagents mentioned above and varying amounts of the antigen. The usual technic is followed and the unit determined by the quantity of antigen that inhibited hemolysis. After this determination the same antigen must be tested with a known negative serum used in place of the positive serum and using double the unit of antigen. This double unit should not inhibit hemolysis of the blood cells. The unit being determined, the antigen is so diluted that 1.0 c.c. will contain the unit.

2. *Antibody.*—The blood serum or cerebrospinal fluid of the syphilitic person. A sufficient quantity of the patient's blood is collected from the lobe of the ear or finger tip, in any sterile vial (best in a Wright's capsule), aseptic precautions, of course, being observed. The blood is then

centrifugalized and the serum used. The spinal fluid is obtained in the usual manner by lumbar puncture.

3. *Complement.*—The normal blood serum of a guinea-pig. The blood from one guinea-pig is required, thus making it necessary to sacrifice one animal for each test. The blood must be used fresh, as the serum loses its complementing value if kept over twenty-four hours. The blood is defibrinated, centrifugalized, and the serum used. If stored, it should be frozen.

4. *Hemolytic Serum.*—The blood serum of a rabbit that has been injected with washed lamb's blood-corpuscles. The rabbit is immunized as follows: The lamb's blood is first obtained, best by cutting its ear and allowing 10 c.c. of blood to run into 30 c.c. of a 1 per cent. sodium citrate solution in normal salt solution. (This will prevent the blood from clotting.) It is then centrifugalized, the supernatant fluid pipetted off, and the blood-corpuscles washed with normal salt solution by repeated centrifugalization and dejection of the supernatant fluid. Five c.c. of the washed blood-corpuscles are injected into the rabbit five (5) or six (6) times at repeated intervals of five (5) days. On about the tenth day after the last injection, blood is taken from the rabbit, centrifugalized, and the serum used. Before using this serum, it is necessary to test its power after being inactivated (heated for three-quarters of an hour at 56° C. to destroy complement). The test is made to determine the minimum quantity of the serum that will hemolyze 1 c.c. of the 5 per cent. suspension of lamb's blood-corpuscles, with 0.1 c.c. of complement (normal guinea-pig serum). Various quantities of the serum to be tested are put in a series of test-tubes with 1 c.c. of the suspension of lamb's blood corpuscles and 0.1 c.c. of the complement in each tube. The tubes are put in the incubator at 37° C., for an hour and then examined to determine the smallest quantity of serum that produced hemolysis. (The proper quantity is usually 1 c.c. of a 1 in 2000 dilution, in normal salt solution. The quantity necessary for the reaction is two minimal units, thus 1 c.c. of a 1000 dilution is used for the reaction.) The dilution used should never be lower than 1:1000. If it happens to be lower it will be necessary to give the rabbit a few more injections of blood-corpuscles, before using its serum.

5. *Lamb's Blood Corpuscles.*—Five c.c. of defibrinated lamb's blood are collected and washed with normal salt solution in the same way as the rabbit's blood. Then a 5 per cent. suspension in normal salt solution is made.

The antigen, the patient's serum and the hemolytic serum must be inactivated (to destroy complement) before using, by heating them for three-quarters of an hour at 56° C. The two sera should be inactivated as soon as made.

The antigen, antibody (patient's serum) complement, and hemolytic serum should each be so diluted with normal salt solution that 1 c.c. of the dilution will contain the necessary quantities needed for the reaction.

*Technic for Performing the Reaction.*—Into a test-tube place 0.2 c.c. of the antigen, 0.2 c.c. of the patient's serum (antibody), and 0.1 c.c. of the complement. Incubate at 37° C. for three-quarters of an hour and then add 1.0 c.c. of the solution of hemolytic serum, containing two minimal doses and 1.0 c.c. of the 5 per cent. suspension of lamb's blood-corpuscles. Incubate the whole for two hours, place in the refrigerator over night, and then note if hemolysis has occurred. If the antibody of syphilis is present in the suspected blood serum, hemolysis will not occur because the complement is "fixed" to the immune body by the aid of the antigen and the reaction is positive. Should the suspected blood serum not contain the specific antibody, hemolysis will occur because there is no immune body to "fix" the complement, therefore causing the hemolytic amboceptor (hemolytic serum), by the aid of the red corpuscles, to fix the complement, producing hemolysis and the reaction is then negative.

The substances employed are subject to many external influences, and it is, therefore, necessary to control their action. The controls made are necessary in order to demonstrate that none of the employed substances alone "fix" the complement, and that the occurrence of either a positive or a negative reaction, when testing a suspected serum, is due to and dependent upon the fixation or non-fixation of the complement by means of the immune body.

The quantity of antigen used for the reaction may have to be either increased or decreased. The controls will indicate when a change is required and the proper quantity necessary is determined by the method given under the preparation of the antigen.

There are many other communicable diseases as measles, mumps, scarlet fever, and whooping cough, besides the diseases due to the attacks of higher parasites, as itch, trichinosis, tapeworm, roundworm, liver flukes, hookworm, etc., which we will, however, not discuss more fully. The suggestions given under the diseases described will also apply, in a measure, to other communicable diseases. Summed up briefly, preventive medicine, direct and indirect, consists of giving heed to the following.

1. Living in accord with the most approved methods of hygiene. This is direct preventive medicine.

2. Treating disease in accord with the most approved modern methods. This is indirect preventive medicine because it protects the well against infection from the sick.

The following table of communicable diseases giving the average period of incubation (also known as latent period), the primary cause, nature of communicability and principle carriers or sources of infection, will be found useful.

| Name of disease. | Incubation period, days. | Primary cause. | Nature of communicability. | Carriers or sources of infection. |
|---|---|---|---|---|
| Anthrax or wool sorter's disease. | 2 | Bacillus anthracis.. | Infectious and contagious. | Cattle, sheep. |
| Bubonic plague. . | 4–6 | Bacillus pestis..... | Infectious..... | Rats, mice, fleas, filth. |
| Asiatic cholera... | 2–4 | Bacillus choleræ. .. | Infectious and contagious. | Flies, polluted water and food. |
| Diphtheria..... | 2–3 | Bacillus diphtheriæ. | Infectious and contagious. | Animals, foods, the sick. |
| Erysipelas..... | 4–6 | Streptococcus pyogenes. | Very contagious to wounds. | Dirt, perhaps flies, mosquitos. |
| Influenza, Grippe | 1–4 | Bacillus influenzæ.. | Infectious, not contagious. | Air, and exposed objects. |
| Glanders..... | 3–5 | Bacillus mallei..... | Contagious and infectious. | Horse and horse-like animals. |
| Gonorrhea..... | 3–5 | Micrococcus gonorrheæ. | Very contagious, not infectious. | All contaminated objects. |
| Mumps..... | 10–16 | Unknown..... | Very infectious..... | Air and the sick. |
| Malaria..... | 6–10 | Plasmodium malariæ. | Infectious, not contagious. | Mosquitos (Anopheles). |
| Relapsing fever.. | 5–6 | Spirochæta Obermeieri. | Infectious..... | Insects, as bed bugs, etc. |
| Measles..... | 8–9 | Unknown..... | Very contagious, also infectious. | Exposed objects. |
| Hydrophobia..... | 20–60 | Unknown..... | Contagious to wounds. | Mad dogs, wolves and other canines. |
| Rubeola, rubella.. | 18 | Unknown..... | Infectious and contagious. | Exposed objects. |
| Scarlatina..... | 2–5 | Unknown, perhaps Protozoa. | Infectious and contagious. | Exposed objects. |
| Small-pox..... | 12 | Unknown..... | Infectious and very contagious. | Exposed objects. |
| Syphilis..... | 14–30 | Spirochæta pallida. | Very contagious, especially to lesions. | Exposed objects. A filth disease. |
| Tetanus, lock-jaw | 2–3 | Bacillus tetani..... | Contagious to lesions only. | Dirt, infected objects of all kinds. |
| Typhoid fever.... | 14 | Bacillus typhosus.. | Infectious and contagious. | Polluted water and food. Flies. |

| Name of disease. | Incubation period, days. | Primary cause. | Nature of communicability. | Carriers or sources of infection. |
|---|---|---|---|---|
| Vaccinia..... | 3–6 | Unknown..... | Contagious by inoculation only. | Cow virus, human vaccinia. |
| Varicella, chicken-pox. | 14–15 | Unknown..... | Contagious..... | Those affected. |
| Whooping cough.. | 8 | Unknown..... | Very infectious..... | Exposure to those affected. |
| Yellow fever..... | 3–4 | Unknown, plasmodium? | Infectious, not contagious. | Mosquitos (Stegomyia.) |
| Leprosy..... | Weeks, months, years. | Bacillus lepræ..... | Infectious and contagious. | The patients. |
| Tuberculosis..... | Weeks and longer. | Bacillus tuberculosis. | Infectious..... | Sputum, milk from tubercular cows. |
| Dengue ..... | 4 | Protozoa?..... | Infectious..... | Mosquito (culex fatigans). |
| Pneumonia..... | 1–2 | Micrococcus pneumoniæ (Diplococcus). | Infectious..... | Carried by persons. |
| Dysentery (bacillary)..... | 8 | Amœba dysenteriæ. | Infectious..... | Polluted water supply. |
| Malta fever..... | 6–10 | Micrococcus melitensis. | Infectious..... | Goats' milk, stings of insects. |
| Beri-beri..... | Months. | Micrococcus?..... | Infectious..... | A tropical disease. |
| Pellagra..... | ? | Aspergillus species.. | Neither infectious nor contagious. | Mouldy foods, corn especially. |

In some diseases the mortality rate is very high, as in yellow fever, beri-beri, tetanus, cholera, plague and leprosy. In others it is low, as in syphilis, gonorrhea, malaria, whooping cough, mumps and varicella. In certain diseases the prognosis is rather uncertain, the mortality rate being high at times and again low, as in scarlatina, small-pox, measles and grippe. Some diseases run a somewhat variably rapid course as pneumonia, diphtheria, spinal meningitis, bubonic plague and Asiatic cholera, ending either in death or recovery. Other diseases, as scarlet fever, measles and diphtheria may have after-effects or sequelæ which often assume a chronic course and may finally result in death. Certain diseases run a regular course which varies but little as to the sequence of symptoms and duration, as typhoid fever (five weeks). Others run a variably chronic course, ending either in death

or recovery, as pellagra and malaria. Some diseases are very persistent, difficult to eradicate from the system, showing certain effects even to the third and fourth generations, as tuberculosis and syphilis. Malaria leaves certain after-effects, as enlarged spleen ("ague cake"), which may persist through life.

Savage races are peculiarly susceptible to certain diseases, as tuberculosis, small-pox, gonorrhea and syphilis and peculiarly enough these diseases did not originate with primitive peoples, but with advanced civilization, though of great antiquity.

## CHAPTER XIV.

## A BACTERIOLOGICAL AND MICROSCOPICAL LABORATORY FOR THE PHARMACIST.

The pharmacist imbued with the proper appreciation of his responsibility in the community should equip a laboratory in which to do the necessary and desirable work in bacteriology and microscopy. The pharmacist, more than any other professional man, has or should have the opportunity to do laboratory work. The significance of bacteriology in pharmacy has been sufficiently set forth in the pages of this book. The details of the microscopical work proper belongs to the field of botany and pharmacognosy and cannot be fully explained here. Every graduate from a reputable college of pharmacy is or should be qualified to use the microscope in the examination of vegetable drugs (crude and powdered), of compound powders, dusting powders, insect powders, starches, meals, flours, including foods of vegetable origin as baby foods, breakfast foods, etc.

The following suggestions will no doubt be useful, though it is not intended to imply that they must be carried out strictly. The laboratory space, location, equipment, etc., can be made to suit individual requirements. The microscopical work should preferably be done in a separate room, not in the bacteriological laboratory, though this is not absolutely essential.

A. *Location of Laboratory.*—It may be in a separate building, as the home, but as a rule a corner room in the pharmacy is best suited for the purpose. This room may be in the basement, or on the first, second or other floor. Do not select a room with a through passage for obvious reasons. It may adjoin a chemical or pharmaceutical laboratory, though it should not be a part of such laboratories. Chemicals and chemical fumes interfere with bacteriological and microscopical work. It should have one door and two or more windows. There must be good light and the environment should be favorable for bacteriological work, for which reason a room in the basement is not, as a rule, desirable.

The walls and ceiling of this room should be absolutely plain and well protected with white enamel paint. The floor may be cement, slate, or hard wood well oiled with boiled linseed oil, or it may be painted, or covered

with linoleum. The entire room (walls, ceiling, floor) should be washed, scrubbed and disinfected from time to time. That is, it should be kept bacteriologically clean.

B. *Furnishings.*—All windows exposed to direct sunlight should have white translucent roller shades. The laboratory should be well supplied with gas; water, both hot and cold, if possible; and means for lighting (gas, electricity, acetylene). There should be just enough furniture and shelving, no more. One table with slate top or lined with linoleum; one stool, shelves for samples, apparatus and reagents. A case for chemicals, cotton, culture media, etc. A case, with lock and key, for samples to be examined. The plumbing must be of the best and the fixtures must be of safe construction. The sink should be large and deep and should be lined with porcelain and supplied with an ample drain board. A hood or ventilator should be provided to carry off steam vapors. Near the table for microscopical work should be a shelf or case for the following works of reference: U. S. Pharmacopœia, National Formulary; National Dispensatory; Sayre's Organic Materia Medica and Pharmacognosy; Culbreth's Materia Media and Pharmacology; Kraemer's Botany and Pharmacognosy; Schneider's Powdered Vegetable Drugs; Winton's (Moeller's) Microscopy of Vegetable Foods; Brundage's Manual of Toxicology; Holland's Urine, Common Poisons and Milk; Muir and Ritchie's Manual of Bacteriology; Official Methods of the American Association of Agricultural Chemists. There are many other desirable reference works, but the above will serve as a nucleus to which additions can be made from time to time. Only the latest editions should be purchased.

C. *Apparatus.*—There will be required:

a. A good simple lens.

b. Compound miscroscope. (Leitz, Zeiss or Bausch and Lomb).
Ocular with micrometer scale.
Oculars, Nos. 2 and 3.
Objectives, Nos. 3, 5, 7 and 1/12 oil immersion.

c. Slides and covers.

d. Section knife or razor, and strop.

e. Polarizer, for the study of starches, crystals, etc. Should be convenient to use. This is very important. The selenite plates which are usually supplied with the polarizer are useful.

f. Thoma-Zeiss hemacytometer with Turck ruling, for counting bacteria, spores and yeast cells in vinegar, jams, jellies and other like substances.

g. Accurately ruled metal or hard rubber millimeter ruler for measuring seeds in fruit products, etc.

h. One Arnold steam sterilizer (copper). A vegetable steam cooker will serve.

i. One hot air sterilizer. The ordinary double walled baking ovens which may be secured from any hardware dealer, will serve the purpose. Cut in a small opening at top for the thermometer.

j. One rice cooker in which to prepare culture media, etc.

k. One small incubator with Reichert thermo-regulator.

l. Centrifuge (electric or water motor).

In addition to the above there will be required the necessary chemicals, reagents, etc., good quality commerical cotton for plugging test-tubes, medium size Petri dishes, flasks (1/2 liter and 1 liter), several evaporating dishes, one or two moist chambers, a quantity of medium size test-tubes, slide boxes, test-tube brushes, dissecting needles, scalpels, labels, pencils, etc. Get the necessary things only. There must be a liberal supply of clean towels. No one but the analyst and his assistants should have access to the laboratory. On entering, the analyst should remove coat and hat and put on a white clean linen apron and coat, such as are worn by soda fountain dispensers. This white suit should remain in the laboratory and should be changed for a clean one as often as may be necessary.

Special equipment and apparatus may be indicated as the work progresses. For instance, it may prove desirable to have an incubator for opsonic work, for the use of physicians, used either by the physicians or by the pharmacist. A water filtering equipment may be installed, likewise a water still. An autoclave may prove desirable. There are matters which must be left to the individual pharmacist. The following is an outline of such work as the pharmacist may do in the microscopical and bacteriological laboratory.

D. *Micro-analytical Work.*—The skilled microscopist should be ready to determine the quality and purity of the following substances:

a. Vegetable drugs, crude and powdered.

b. Spices and condiments, whole, ground and powdered. Prepared spices and condiments.

c. Coffee, tea, cocoa, chocolate, confections, candies.

d. Tobacco, including smoking tobacco, cigars, cigarettes, snuff.

e. Compound powders, pharmacopœial and others.

f. Tablets, pills, simple powders.

g. Meats; raw, cooked, canned, sausage meats, mince meats, etc.

h. Dairying products as milk, cream, cheese, butter, ice creams, cream fillers.

i. Cosmetics, dusting powders, insect powders.

j. Cattle and poultry powders.

k. Starches, dextrins, sausage meat binders (starchy).

l. Vegetable foods; as jams, jellies, fresh, pickled, cooked, canned and preserved.

m. Flours and meals.

n. Breakfast foods, baby food, invalid foods.

o. Breads, cakes, pies, crackers, etc.

p. Catsups, tomato pastes, etc.

q. Macaroni, spaghetti, noodles, etc.

r. Nuts, and nut-like fruits.

s. Cloth material, textile fabrics generally, cordage, papers, etc.

It is assumed that the pharmacist has had the necessary training to undertake the microscopical examination of the substances above classified, with the aid of such standard works of reference as may be required. The micro-analyses should also include:

1. Gross and net weight determination of all samples that require it, for which purpose an accurate balance is necessary.

2. Moisture determinations of such substances as may require it. There should be no difficulty in constructing the necessary apparatus for making moisture determinations.

3. Ash determinations of substances which require it. This calls for a special equipment including a platinum dish, ignition furnace with burners, etc.

4. Use of special tests, as sublimation tests for benzoic acid and salicylic acid, the hand wheat gluten test, Bamihl gluten test, Grahe's cinchona test, color reaction tests for boric acid, salicylic acid, morphine, and opium; tests for phytosterol and cholesterol crystals, etc., etc. These and other tests are explained in the several reference works cited above. In the examination of liquids or semi-liquids as wines, beers, cider, vinegar, milk, cream, sewage, extracts, tinctures, etc., a centrifuge is desirable.

E. *Bacteriological Work.*—The pharmacist should be prepared to do the following work in the bacteriological laboratory.

a. Prepare culture media for use of physicians, as may be required.

b. Prepare sterile throat swabs for the use of physicians.

c. Prepare stains and do staining for physicians, as may be required.

d. Make bacteriological determinations of milk, jams, jellies, impure drinking water, vinegar, wine, sera, vaccines, antitoxins, contaminated foods and drinks, sewage, etc.

e. Sterilize pharmaceuticals, surgical supplies, etc.

f. Assist the physician in opsonic work, as may be arranged or agreed upon.

g. Do bacterial culture incubation work for the physician, make subcultures, Wassermann reaction for syphilis, etc.

h. Filter and sterilize drinking water to be supplied to customers.

The above outline is intended as a suggestion only. Experience and opportunity will determine what can actually be done.

The following is a diagram of a bacteriological and microscopical laboratory:

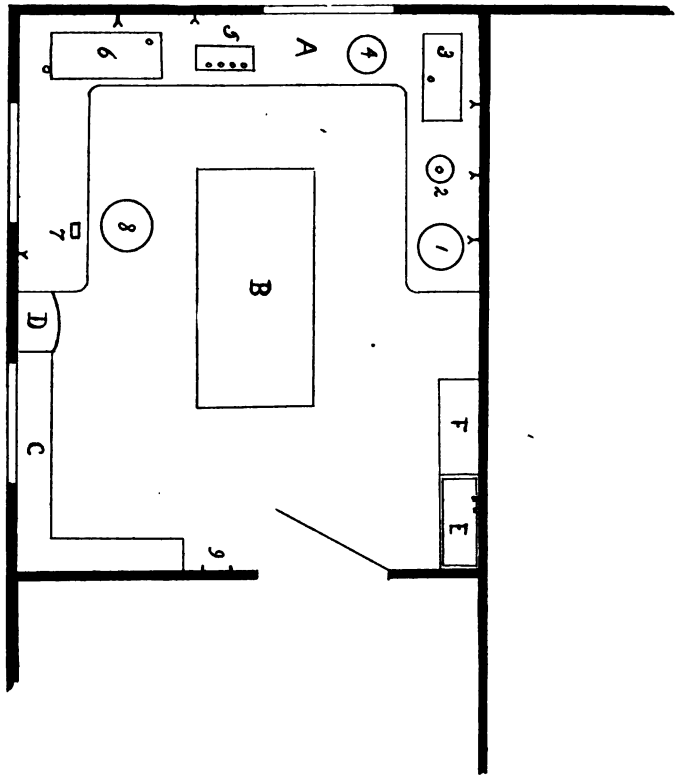


FIG. 86.—Plan of bacteriological and microscopical laboratory, using corner room in the pharmacy. (Scale 4 feet to the inch.) *A*, shelves on three sides of the room. *B*, work table. *C*, cases and shelves for reagents, chemicals, glassware, etc. *D*, case for reference books. *E*, sink. *F*, drain board. 1, Arnold steam sterilizer; 2, hot water filter; 3, hot air sterilizer; 4, rice cooker; 5, opsonic incubator; 6, incubator; 7, compound microscope; 8, stool; 9, hat and coat hooks.

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